TOPLEY AND WILSON'S PRINCIPLES OF BACTERIOLOGY AND IMMUNITY

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THIRD EDITION

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CONTENTS

VOLUME II

PART III

INFECTION AND RESISTANCE

CHAPTER		PAGE
42.	TYPES OF IMMUNITY.	971
43.	THE MEASUREMENT OF IMMUNITY REACTIONS IN THE LIVING ANIMAL.	980
44.	THE MECHANISMS OF BACTERIAL INFECTION	1002
45.	THE MECHANISMS THAT HINDER OR PREVENT THE ACCESS OF BACTERIA TO THE TISSUES	1019
46.	THE MECHANISMS OF ANTITOXIC IMMUNITY	1029
47.	THE MECHANISMS CONCERNED IN SPECIFIC ANTIBACTERIAL IMMUNITY	1034
48.	THE AGGRESSIVE ACTION OF BACTERIA	1068
49.	THE NATURAL ANTIBODIES: THEIR NATURE, ORIGIN AND BEHAVIOUR	1075
50.	THE ANTIBODY-FORMING APPARATUS AND ITS REACTIONS	1101
51.	ANAPHYLAXIS, HYPERSENSITIVENESS AND ALLERGY	1136
52.	CERTAIN NON-SPECIFIC MECHANISMS IN GENERAL IMMUNITY	1173
53.	LOCAL IMMUNITY	1180
54.	THE INFLUENCE OF DIET, FATIGUE, CHANGES IN TEMPERATURE AND HUMIDITY, CHEMICAL AND CHEMOTHERAPEUTIC AGENTS AND OTHER FACTORS ON GENERAL OR LOCAL IMMUNITY	1190
55.	IMMUNITY IN VIRUS DISEASES	1225
56.	HERD INFECTION AND HERD IMMUNITY	1245

PART IV

THE APPLICATION OF BACTERIOLOGY TO MEDICINE AND HYGIENE

57.	ACTINOMYCOSIS, ACTINOBACILLOSIS AND RELATED DISEASES	1269
58.	SWINE ERYSIPELAS, ERYSIPELOID, MOUSE SEPTICÆMIA, AND INFECTIVE MONONUCLEOSIS OF RABBITS	1283
59.	TUBERCULOSIS	1289
60.	LEPROSY RAT LEPROSY, AND JOHNE'S DISEASE	1358
61.	DIPHTHERIA AND OTHER DISEASES DUE TO CORYNEBACTERIA	1368
62.	GLANDERS AND MELIOIDOSIS	1408
63.	CHOLERA	1418
64.	MENINGITIS	1431
65.	GONORRHŒA	1454
66.	SCARLET FEVER, AND OTHER DISEASES DUE TO HÆMOLYTIC STREPTOCOCCI	1462
67.	PYOGENIC AND WOUND INFECTIONS	1490

PRINCIPLES OF BACTERIOLOGY AND IMMUNITY

VOLUME II

PART III

INFECTION AND RESISTANCE

CHAPTER 42

TYPES OF IMMUNITY

THE term *immunity*, used as we use it here, needs definition. As plain English, it does not mean what it says. This does not matter very much because, as Whitehead points out so aptly in his *Introduction to Mathematics*, the scientist shares with Humpty Dumpty the privilege of paying words extra and making them mean what he likes. But if we are to avoid confusion we must at least give our words definite orders and see that they are obeyed. It happens that the state suggested by the word that we have chosen as a generic label for the phenomena we wish to study is one about which we know very little, except that it exists. We have indeed not bothered very much about it. Complete natural insusceptibility to infection removes the relationship between the particular host and parasite concerned from our field of interest, except in so far as we can learn from any instance of this kind something of the genetic laws that determine the transmission of this innate and complete resistance. Our main business is with those interactions between parasite and host that are characterized by a fluctuating equilibrium, and with the factors that shift this equilibrium, so that sometimes the parasite, sometimes the host, gains the upper hand. *Resistance* would be a better word than *Immunity*; but *Immunity* will serve well enough so long as we make it quite clear that we are using it to denote the resultant of two opposing systems, and that this resultant can assume any value from zero to infinity.

And our name misleads a little because of its associations. It happens to have been frequently applied to the data obtained by a particular kind of technique, and there has been a tendency to confuse technique with subject-matter. The special methods devised by the immunologist—if he must accept the unpleasing name that custom has given him—are applicable to problems with which he has no concern; while the field proper to his study extends far beyond the limits that would be imposed by any definition in terms of the particular technique employed. The student of immunity is concerned with all data relating to the mechanisms involved in infective disease, by whatever means they are obtained. Any account given at the present time must stress unduly a particular set of phenomena, simply because they are the only phenomena that have as yet been adequately studied; but this is a historical accident that will be remedied by time.

The phenomena that present themselves for study are derived from the most

diverse sources. Some come to us from epidemiology, using that term in its widest sense. Under natural conditions different animal species show a widely differing incidence of certain infections. Thus, anthrax is in the main a disease of herbivora. Of the animals dying of anthrax in this country in 1914, 733 were cattle, 5 were sheep, 32 were swine and 25 were horses. In Australia and South America sheep are more commonly affected; but Algerian sheep are stated to be highly resistant. Tuberculosis is one of the commonest natural infections of man and cattle. It is common in pigs and in fowls. It is relatively uncommon in sheep, goats, horses and dogs. It is stated to be very uncommon as a natural disease in rabbits, guinea-pigs, rats and mice. Among mankind there are well-marked racial differences in its incidence and severity. In eight of the great cities of the United States in 1920 the mortality from tuberculosis among the white population varied from 0·794 to 1·216 per 1,000 living; among the coloured population it varied from 2·855 to 4·205 per 1,000. Such instances could be multiplied *ad nauseam*.

The epidemiologist also records differences in the incidence and fatality of various infective diseases at different ages, suggesting in many cases an increase in resistance with age. He notes also that repeated attacks of the same infective diseases are in some instances very rare, as in measles, or smallpox, or diphtheria, or typhoid fever, while in others they are relatively common, as in influenza, or pneumonia, or the common cold.

Other data come to us from clinical medicine in the narrower sense. Infections that, in their usual course, progress slowly to death or recovery sometimes assume a fulminating form. Such a protean infection as tuberculosis shows the widest diversity in the varying prominence of its local and general manifestations. And, in any infective disease, why do some patients die and others recover?

For the immunologist these are crude data that require analysis. Using his own methods he re-examines the phenomena presented by natural infection. Sometimes he transforms the picture they present. He finds, for instance, that the recorded frequency of natural tuberculosis in various animal species does not in all cases reflect their relative resistance to experimental infection (see Chapter 59). He discovers also that there may be a diversity of immunological states within a single clinical syndrome. This is true of tuberculosis; there are different types of the tubercle bacillus, and a given host species is more resistant to one type than to another. It is true of lobar pneumonia. For the immunologist this is not one disease but several, each caused by a significantly different type of pneumococcus; and he notes that the statement that second attacks of pneumonia are not uncommon may belong to the Baconian category of a truth that has in it a mixture of a lie. Again he finds that enteric fever is not one but many; and his attempts at interference are planned accordingly.

Another important change that the immunologist makes in the clinical and epidemiological picture is in regard to the character and extent of the association between any given parasite and the host species that it infects. He finds that the real range of interaction includes states of equilibrium in which the host shows no overt signs of disease. Whether we call all these conditions latent infections, or refer to many of the hosts as healthy carriers, matters little. No hair-splitting definition will help us much. The significant thing we have learned is that in some infections, such as measles, contact between a previously uninfected host and the virus of the disease usually results in a clinically characteristic attack, so that the epidemiological picture gives us a reasonably adequate description of

the biological association, while in other diseases, such as cerebrospinal meningitis or poliomyelitis, the clinically diagnosable cases form so small a fraction of the total number of infected persons that they might almost be regarded as occasional accidents in an association that, in its modal form, induces no such serious effects.

In re-examining the data that he receives from the field and from the ward, the immunologist relies in large part on experiments on animals. When he tries to interpret his data he is forced to rely almost entirely on this fundamental method of study. He cannot advance without some simplification of his problems, some control, at least, over the innumerable variables that determine the incidence and results of infective disease as it occurs in nature.

By such experiments he has found that he can increase the resistance of animals by infecting them with sublethal doses of a given pathogenic organism, or by injecting an organism that has lost its power to kill—though here he was forestalled by Jenner's experiment of vaccination in man—or, more safely and conveniently, by injecting dead organisms or their products. From analogy, and from the fact that he can demonstrate similar changes in the tissue fluids, he concludes that this artificial immunization in animals is essentially similar to the natural immunization that occurs during an attack of an infective disease. Since the tissues of the naturally infected or artificially immunized host play an active part in bringing about this increased resistance, he calls it *active immunity*. In some cases he finds that he can transmit this resistant state to a normally susceptible animal by injecting into it the serum of another animal that has been rendered immune. Since the tissues of the recipient appear to play a relatively passive part, he calls this *passive immunity*. He also finds that immunity of this kind is sometimes transmitted naturally from a mother to her young, either by the passage of the protective substances *via* the placental vessels, or by their ingestion during the first days or weeks of life in the colostrum. This he calls *congenital passive immunity*.

And so, if he has a taste for classification, the immunologist can draw up some such list as this:

1. Innate Immunity.

2. Acquired Immunity.
   - (*a*) Active.
     - (α) Naturally acquired.
     - (β) Artificially induced.
   - (*b*) Passive.
     - (α) Naturally acquired (congenital).
     - (β) Artificially induced.

Innate, or Genetic, Immunity.

Of innate as opposed to acquired immunity, little need here be said. We have noted that different animal species may display wide differences in their resistance to various bacterial parasites, or to their toxins. This species immunity is of great practical importance in relation to the communicability of infective disease from animals to man, or from one animal species to another.

There can be no doubt that differences in innate resistance also occur within any animal species, one individual differing from another in this biological character as in any other. Of the extent of these differences and of the laws that govern their inheritance we as yet know very little. Within recent years a number of workers have attempted to study this problem by direct experiment, and their results indicate that it is possible to increase or lower the average resistance of a given strain of rats, mice or other experimental animals, by selective breeding. (See for instance Webster 1923, 1924*a*, *b*, 1925, 1933*a*, *b*, Pritchett 1925, 1926*a*, *b*, Lambert and Knox 1928, Irwin 1929, 1933, Irwin and Hughes 1931, 1933, Lambert 1932, Schott 1932, Gowen 1933, Gowen and Schott 1933*a*, *b*, *c*, Schütze *et al.* 1936, Hill *et al.*, 1940, Lurie 1941, Gowen and Calhoun 1943).

Experiments of this type are, however, subject to great technical difficulties. The obvious method of obtaining a strain of animals with a high genetic resistance is to infect an adequate sample with the bacterium under study, breed from the survivors, and repeat the process through several subsequent generations. This plan has, in fact, been followed by some of the workers referred to above ; but it is clearly open to serious sources of error. In testing the resistance of our original generation we shall certainly alter it, and the effect of this alteration will not be confined to the parent animals. The surviving females will pass on a temporary passive immunity to their young ; and, since most of the species commonly employed in such tests attain sexual maturity within a few months at most, this congenital passive immunity may persist until the F_1 generation are tested. A much more serious source of error is that the survivors will often be carriers of the organisms with which they were infected. Either the male or female parent may thus infect the young, inducing in them an acquired active immunity, or adding it to the congenital passive immunity already present. If this source of error is avoided, by mating the original sample of animals at random, and testing them after they have been separated from their young, the number of animals that must be employed, and the resulting labour, are enormously increased ; since it will only be by chance that both parents of any one litter will be found to possess a resistance above, or below, the average.

The papers referred to above describe various attempts to overcome, or minimize, these technical difficulties ; and they are very fully discussed in a recent monograph by Hill (1934). His conclusion is that, while possible errors of the kind we have mentioned render doubtful many of the observations that have been recorded, a presumptive case has been established for the existence of genetic differences in resistance within a breed or species, as well as between breeds or between species. In regard to the degree of these genetic differences, within a strain or breed, it is much more difficult to arrive at any just conclusion. Some of the differences recorded have, in fact, been quite trivial ; but a few have been of a relatively high order.

As an example of the latter we may quote results recorded by Webster (1933*b*). By selective breeding, carried out through several generations, he was able to obtain a particularly resistant, and a particularly susceptible strain of mice, both originally derived from the strain that has been bred for many years at the Rockefeller Institute. The organism against which they were tested, in each successive generation, was *Salm. enteritidis*. The susceptible strain finally developed showed a mortality of 85 to 95 per cent. when infected with this organism, while the resistant strain showed a mortality of the order of 15 per cent. It is of interest to note that the difference in resistance between these two strains was non-specific, in the ordinary bacteriological sense. Thus the susceptible strain showed a mortality of 63 per cent., when tested against *Past. septica*, 80 per cent. when tested against Friedländer's bacillus, and 38 per cent. when tested against the pneu-

mococcus, while the corresponding figures for the resistant strain were 35 per cent., 45 per cent. and 16 per cent. respectively. On the other hand the susceptible strain showed a mortality of 40 per cent., when tested against the virus of louping-ill, as compared with a mortality of 60 per cent. in the resistant strain; so that the genetic factors concerned, whatever they may be, do not seem to operate against all types of infection, though they are operative against many.

Webster (1937) was later able to develop three strains of mice which he regarded as genetically stable in their reactions to *Salm. enteritidis* and to the virus of St. Louis encephalitis—one susceptible both to bacterium and virus, a second susceptible to bacterium but resistant to virus, and a third resistant to virus but not to bacterium. The resistance displayed by the animals resulting from various cross-breeds of these strains were in accord with the hypothesis that susceptibility to the two infective agents is regulated by two distinct sets of inherited factors (see also Church 1939).

Schütze, Gorer and Finlayson (1936), who have made tests on Webster's resistant and susceptible strains, record rather different results. The resistant strain showed a lower mortality than the susceptible when tested against *Salm. enteritidis* or *Salm. typhi-murium*; but when the two strains were tested against the pneumococcus, or against *Past. septica*, there was no significant difference in their behaviour. The discrepancy between these results and those recorded by Webster may be due to the fact that Schütze and his colleagues infected their mice by intraperitoneal injection, while Webster used the intranasal route. In Schutze's experiments both strains were equally susceptible to the virus of louping-ill.

We are still largely ignorant of the defence mechanisms whose effectiveness is determined by genetic constitution. Gorer and Schütze (1938) found some evidence that in a strain of mice resistant to *Salm. typhi-murium* the specific antibody response to the "H" antigen was better than in a susceptible strain, but this relation did not hold for "O" antigen, nor for the "H" or "O" antigens of *Salm. enteritidis*. Lurie (1941) on the other hand, working with inbred strains of rabbits, of low and high resistance, demonstrated that both the degree of cellular response to invasion by the bovine tubercle bacillus, and of skin response to the injection of heat-killed bacilli, was higher in the tuberculosis-resistant strains. (see p. 1299). In inbred strains of mice selected for resistance to *Salm. typhi-murium* the degree of resistance was found by Gowen and Calhoun (1943) to be correlated with the mean number of leucocytes circulating in the blood. A large number of circulating leucocytes, however, is probably a manifestation of a general not of a specific resistance to the organism, for, as Reich and Dunning (1941) showed in rats, the numbers of leucocytes may be correlated with general fitness for survival.

Severens, Roberts and Card (1944) studied the defence mechanism in two breeds of hen, which as chicks aged 1–10 days were respectively susceptible and resistant to infection with *Salm. pullorum*. All the demonstrable differences in the reactions of the two breeds were connected with lymphocytes. Age for age, the number of circulating lymphocytes, the rate of their normal increase after hatching, and the number present in the tissues of the spleen, were all greater in the resistant breed of chick. Moreover, splenectomy reduced both resistance and the circulating lymphocytes in the resistant chicks, but had little effect on the susceptible. The resistance was not due to a greater bactericidal power of the blood, although blood lymphocytes were observed to be phagocytic. It is suggested that the greater availability of lymphocytes may have increased the resistance by providing large numbers of precursors for macrophages in the tissues of the resistant chicks.

It would be unwise to expect that an increase in strain resistance would be determined by a change in a single group of mechanisms. For example, Hill, Hatswell and Topley (1940) bred mice from the survivors of a certain dose of the endotoxin of *Salm. typhi-murium*, and repeated the procedure for ten generations. The tenth generation was highly resistant to the toxin as compared with control unselected mice, but in spite of this it had not acquired any resistance to infection with the living bacillus.

Acquired Immunity.

It is with acquired immunity that we are here mainly concerned; and our interest lies particularly in those reactions that we can induce, and study, in the laboratory. Before discussing these mechanisms in any detail, it will, however, be well to consider briefly the general difference in behaviour displayed by immune and susceptible animals.

Grades of Immunity.

We have noted that the kinds of immunity in which we are most interested are seldom absolute. To keep our ideas clear and precise we may consider briefly the grades of immunity that can, in fact, be demonstrated, remembering always that they shade into one another by imperceptible degrees.

Fig. 236 gives, in diagrammatic form, a rough classification that will be sufficient for our immediate needs. It represents an infection of the invasive type, associated

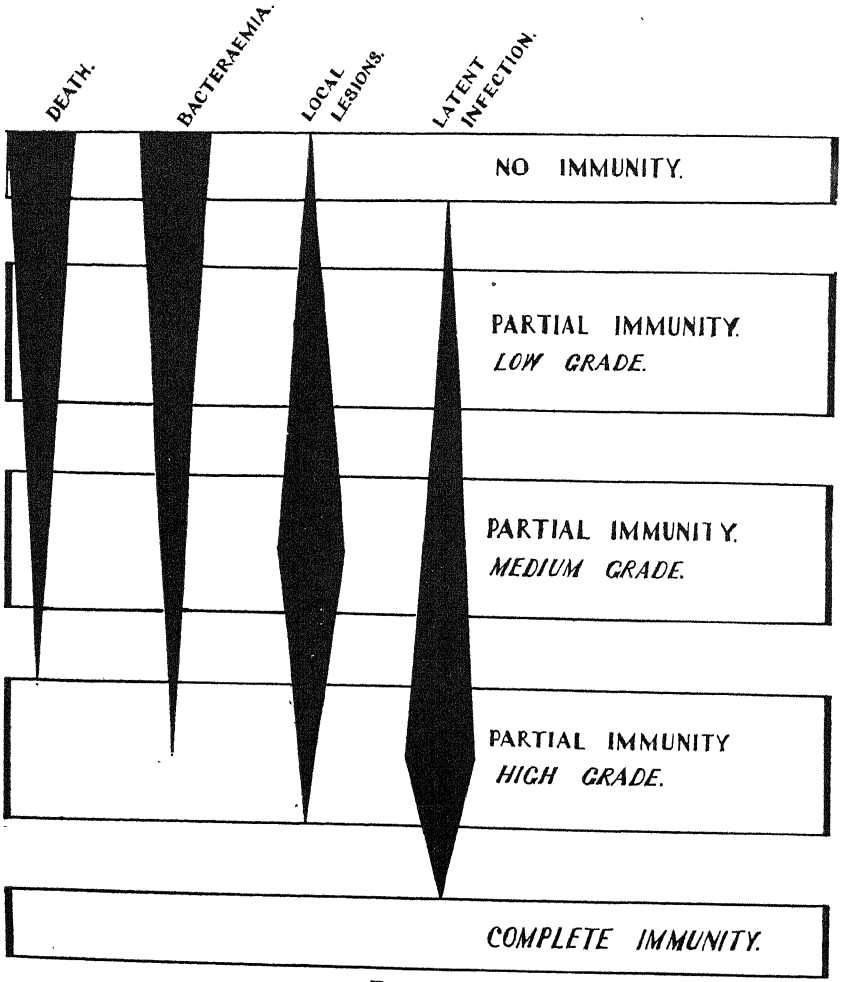


FIG. 236.

with a bacteræmia (an invasion of the blood stream by micro-organisms) as well as with local lesions; but it can easily be modified to meet the case of a toxæmic infection.

The width of the black wedge-shaped areas at any level may be taken to represent the chance of death, and the degree, or frequency, of bacteræmia, of local lesions, and of latent infections, in a sample of individuals possessed of approximately the same grade of immunity.

Thus, starting with the completely susceptible, we may assume that all, or almost all, infected individuals, will develop an acutely fatal bacteræmic infection. Local lesions will be infrequent, and minimal when they occur. There will be no latent infections.

Passing to our next arbitrary grade—partial immunity of low degree—we find fatal bacteræmic infections becoming less common, local lesions more frequent and more pronounced, and a small but increasing number of latent infections.

With partial immunity of a medium grade we find bacteræmia and death much less frequent, local lesions common and relatively extensive, and latent infections increasing in frequency.

With partial immunity of a high grade, death no longer occurs, bacteræmia is infrequent and, when it occurs, is slight and transient. Local lesions are becoming much less frequent and, when they occur, much less extensive. Latent infections reach a maximum frequency and then begin to decline.

Finally we reach the ideal—perhaps never fully attained—of complete or solid immunity. The host is entirely impervious to all attacks of the parasite.

It will not have escaped attention that the grades of resistance that we have labelled as partial immunity are compatible with severe and often fatal infections, and that many infective diseases in their common clinical form might be regarded as occurring in partially immune persons. This view is almost certainly the right one. The syndromes that normally characterize such diseases as typhoid fever, or lobar pneumonia, are expressions of partial immunity. They would not occur in a completely susceptible population attacked by a fully virulent parasite. The latter proviso is, of course, necessary. We may logically use the term immunity to express the relation of any given host to any given parasite, but events are determined by a balance—the balance between the virulence of the parasite and the resistance of the host.

The conception that infective disease, as we usually see it, is an expression of partial immunity, and that minor increases in immunity tend to increase the frequency of milder infections, is of sufficient importance to justify a few illustrative examples, drawn from experimental data.

It was noted by Smith and Moore (1892) that normal and actively immunized rabbits respond very differently to the subcutaneous inoculation of *Pasteurella septica*. Normal rabbits develop a rapidly fatal septicæmia. Immunized rabbits may develop large local abscesses, but show much less tendency to succumb to an acute generalized infection. More recent studies by Jones (1924) afford a good illustration of active immunity in all its grades. The normal rabbit is highly susceptible to *Pasteurella septica*, dying of a generalized septicæmia after the subcutaneous or intratracheal inoculation of 0·01 ml. of a broth culture. In the latter case pulmonary lesions are slight or absent. In rabbits partially immunized by a single injection of a killed vaccine a similar intratracheal injection of living culture is followed, in most cases, by a severe and fatal pneumonia, usually associated with a suppurative pleurisy and pericarditis. In rabbits immunized by repeated doses

of vaccine, intratracheal injections of living culture are usually without effect, while subcutaneous injections lead to the formation of localized abscesses followed by recovery.

Similar phenomena have been recorded in other bacterial infections. Thus Wadsworth (1904) found that the intratracheal injection of virulent pneumococci into normal rabbits was followed by a rapidly fatal septicæmia without local lesions, while similar injections in partially immunized rabbits were often followed by a characteristic pneumonic consolidation.

Differences of the same kind have been observed when strains of pneumococci of varying virulence have been injected intratracheally into normal rabbits. Thus Gaskell (1927) found that strains of high virulence gave rise to a rapidly fatal septicæmia without obvious pulmonary lesions; those of lower virulence to a fatal lobar pneumonia; those of still lower virulence to a patchy lobular consolidation, grading into an entire absence of reaction as the strains employed approached the region of complete avirulence.

The Relation of Immunity to Epidemiology and to Clinical Medicine.

Immunity is essentially an applied science. Its primary data are drawn from clinical medicine, and its outstanding achievement is that it enables us to do things, to interfere intelligently in the natural course of infective disease.

It must, therefore, remain in the closest touch with the field and with the ward. Its hypotheses, based largely on experiments in the laboratory, must stand their trial under field conditions. And here we are faced with a difficulty that is not shared by the more exact sciences. The relations of physics to the mechanical industries are superficially of the same kind as those of immunology to medicine, but actually they are very different. If a result obtained in the physical laboratory suggests a useful application to wireless reception or to aviation, or to any other practical and utilitarian end, it can be tried out with a reasonable certainty that its actual effect will be sufficiently obvious. It may succeed at once, or fail decisively at once, or succeed after adjustment; but in any case definite answers will come back from the field to the laboratory, and both parties to the transaction will learn something from their success or their failure. In medicine this is not always the case, simply because the practice of medicine is a much more difficult thing than any kind of mechanical industry, or indeed than any industrial process at all.

It may be quite easy to tell when an immunological procedure is an unqualified practical success. It is much more difficult to tell whether it is a partial success, or an unqualified failure. And this cause of confusion, which in truth affects the immunologist in the laboratory almost as much as the physician in the ward, is so important that it deserves a separate chapter.

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CHAPTER 43

THE MEASUREMENT OF IMMUNITY REACTIONS IN THE LIVING ANIMAL

It is impossible to understand the present position of immunology, with its mixture of established fact, half-knowledge, hopeful guessings and frank bewilderment, without an adequate grasp of the difficulties involved in measuring immunity reactions in the living animal, and in assessing the significance of such measurements when they have been obtained.

That these difficulties should have been overlooked by the pioneers is not altogether surprising. The effects demonstrated in many of the earlier experiments were so dramatic as to need no exact measurement to establish their significance. The need for standardizing antitoxic sera did, indeed, provide an obvious opportunity for developing adequate biometrical methods; but the need was not realized, for reasons that will become apparent later in this chapter, and the opportunity was allowed to pass. As new methods of immunization were developed and tested in the laboratory, in the ward and in the field, the publication of violently conflicting reports as to their efficacy showed that something was amiss; but the remedy was not obvious, at least to the immunologist. The barriers that separate the different departments of science are not easy to break down, especially when the roads by which these departments are entered diverge widely in their course and are hedged by very different intellectual disciplines. It was natural enough that the laboratory worker and the clinician should show little eagerness to learn and apply the methods devised by the statistician. But the result has been a quite unnecessary amount of confusion; and the confusion is likely to persist so long as the need for such methods is ignored. It will not, therefore, be extravagant to devote a chapter to a brief discussion of the ways in which some of our present difficulties may be overcome—by avoiding the need for statistical methods where this is possible, and by using them intelligently when they cannot be escaped. It will be necessary to refer to many of the reactions that will be discussed in detail in later chapters; but, for the moment, their exact nature and significance does not concern us—we are regarding them merely as things that can be measured.

It is obviously beyond the scope of this book to describe, even in outline, the technical details of statistical measurement or even to give an easy and satisfactory account of the principles upon which such measurement is based. The student is advised to refer to one of the excellent textbooks available, such as those of Yule and Kendall, of Pearl (on the more purely medical side), of Fisher (on the laboratory and experimental side), and for a general conspectus of the subject to the smaller primers of Woods and Russell or of Hill. We must confine ourselves

here to a brief discussion of some general principles, with illustrative examples drawn from a few immunological problems.

The cause of our troubles is sufficiently obvious. It has been set out very clearly by Greenwood (1924) and by Yule (1924). The worker in the more exact sciences can reduce his uncontrollable variables to a minimum, or eliminate them altogether, and can so obtain constantly reproducible results. We cannot. In experiments on living animals, and still more in assessing the value of therapeutic or prophylactic procedures in man, we cannot exclude the interplay of factors about which we know little, except that they are certainly very numerous and may be very important. We cannot avoid an element of randomness in our observations; but, if we have planned our experiments wisely and fortune has been kind in eliminating major disturbing factors that we could not have foreseen, this randomness will be of the kind that determines the number of heads and tails observed in a hundred tosses of a coin. The effect of such random variation can be calculated, and hence allowed for; it is the assessment of the necessary allowance in any particular case that is our main concern.

It will simplify discussion to consider in turn a series of problems of the kind that are continually presenting themselves for solution.

THE MEASUREMENT OF RESISTANCE

To take the most obvious problem, though not the simplest, how can we measure the resistance of an animal to a particular parasite or to its toxin? Suppose first that we wish to measure directly the resistance of the animal as a whole, taking death or survival as our test. Then it is only under quite exceptional conditions that we can obtain *any* measurement on a single animal. If we are dealing with a very powerful toxin that kills rapidly, within a few minutes or hours, it is sometimes possible to give repeated and increasing doses at short intervals and so to measure approximately the amount which is necessary to cause death. But if our toxin acts more slowly, or if we are dealing with a living bacterium, this method cannot be employed. If we start with a small dose and the animal fails to die during the period—usually measured in days—within which we should expect death to occur, we cannot test it again by giving a larger dose, because our first dose will almost certainly have changed its resistance. It is not, from our present point of view, the same animal as before. If we start with a large dose and the animal dies, we do not know how it would have reacted to a smaller dose; if it lives, we know that it is resistant at that particular dose level, but we do not know and can never discover how it would have reacted to doses larger still. It is clear that, if we take death or survival as our test, we must deal with groups not with individuals. This condition is, in truth, usually implicit in the question we want to ask. We are not often interested in the resistance of a particular mouse, or guinea-pig, or rabbit, as such. We are almost always regarding the individual as a representative of a class—normal, or treated in some way that may, we think, have altered resistance to a particular bacterium or toxin—and we are none of us naïve enough to believe in a standard guinea-pig, even if we confine ourselves to animals of a given breed, sex, age and weight. What we sometimes fail to realize is that we must, in most cases, test large numbers of animals before we can regard our result as representing truly the average behaviour of the class from which those animals are drawn.

There are occasions, however, on which we do want to measure the resistance of an individual man or animal; and, before discussing the methods to be adopted in measuring the resistance of groups, we may consider whether there is any way of solving this particular problem.

The Measurement of the Resistance of an Individual.

We cannot, as we have seen, obtain a direct measure of the resistance of any individual animal as a whole; but we can sometimes measure the resistance of a particular tissue, provided that it is possible so to localize the effect of any one inoculum that the reactions to several inocula can be observed simultaneously. This method is commonly exploited in the determination of skin sensitiveness to bacterial toxins. The local effect of a small intradermal inoculation—0·2 ml. is a convenient amount—is limited to the near neighbourhood of the site of injection. By spacing injections so as to leave a few centimetres between them it is easy to test several dilutions of a toxin on a comparatively small area of skin. In this way we can obtain an approximate measure of the smallest dose of toxin that will yield a characteristic reaction, and we can regard this as a measure of resistance—the smaller the dose that will produce such a result the less the resistance of the person or animal under test. Sometimes we rely on a different kind of skin test—the allergic—in which a positive reaction indicates not susceptibility but resistance, showing an increased energy and rapidity of response to a particular bacterial product.

There is another way in which we may attempt to estimate individual resistance—the demonstration and measurement of specific antibodies in the blood. In some cases we may measure the protective power of the serum against a toxin, or a virus, or occasionally against a living bacterium; but this involves further tests on animals, so that we are faced with the same problem as before. Alternatively we may make use of *in vitro* antibody reactions—agglutination, complement fixation, the bactericidal power of the whole blood, and so on. But our measurement of resistance is now becoming very indirect, and we must watch our assumptions with corresponding care.

All these indirect methods of measuring *individual* resistance are, in fact, dependent on a preliminary knowledge of *average* behaviour. We cannot accept our skin tests, antitoxin titrations, agglutination reactions, and so on, as evidence of immunity until we have shown that such a correlation exists; and, to establish the existence of this correlation, we must study the behaviour of groups.

The Measurement of the Average Resistance of a Group.

Let us start with the simplest problem. How can we determine whether a given procedure has made any difference at all in the resistance of a group of animals, even without measuring how large that difference is? If the increase in resistance is of a very high order, our task is easy. We have only to administer to our treated animals a large dose of bacteria or toxin, which experience has shown to be certainly fatal to all untreated animals of the same species, age, weight, etc., and note that the treated animals survive. But we shall be wise to make very sure about our "certainly fatal" dose, especially if our group of treated animals is small. We shall, indeed, always test a few untreated controls to guard against any gross experimental error.

But the increase in resistance may not be of this dramatic kind. Our treated

animals may be unable to withstand a dose of bacteria or toxin that will kill *all* untreated controls, though the mortality in treated and untreated groups may differ appreciably. Our problem is to determine whether this observed difference is likely to have occurred as the result of chance.

One method is to calculate the chances directly by straightforward but laborious arithmetic.

Suppose that we know, from an extended experience, that a particular experimental procedure causes a certain average death rate among large groups of animals. Let us express this death rate as p, using a decimal instead of a percentage notation (*i.e.* expressing a 50 per cent. death rate as 0·5). Then p will also be the chance of any particular animal dying. We should, in other words, expect to get death and recovery equally often if we made a series of tests, each on a single animal. Any animal under test must either live or die, so that, if the chance of death is p, the chance of life is q, where $q = 1 - p$. Expressed in figures, if the chance of death is 0·3 the chance of life must be 0·7; or, to revert to our more familiar percentages, if the percentage mortality is 30 the percentage survival must be 70.

If we know the true average mortality rate, or chance of death, how often are we likely in any single test to meet with a death rate that differs from the average death rate by any specified amount?

Suppose we make an experiment on two animals. Let us call them A and B. Using the notation referred to above, A's chance of dying is p. If A dies, B may either live or die; and B's chance of death is also p. So the chance of both A and B dying is $p \times p$ or p^2. The chance of both A and B living is clearly q^2. The chance of A living and B dying is $p \times q$; and this is also the chance of A dying and B living. So the chances of one animal dying and the other living, irrespective of which does which, is $2pq$. We have therefore:

Chance of two deaths	p^2
Chance of one death	$2pq$
Chance of no death	q^2

These are the successive terms of the expansion of the expression $(p + q)^2$, and we can deal in exactly the same way with a group of any size, by substituting the number of animals in the group for the 2 in the expression given above. For instance, if we had four animals in a group, our expression would become $(p + q)^4$ and the chance of getting 4, 3, 2, 1 or 0 deaths in any single test would be p^4, $4p^3q$, $6p^2q^2$, $4pq^3$ and q^4 respectively.

To take a numerical example, if our p were 0·5, representing an average 50 per cent. mortality, our q would also be 0·5, and our expression would be $(0{\cdot}5 + 0{\cdot}5)^2$ for 2 animals and $(0{\cdot}5 + 0{\cdot}5)^4$ for 4 animals. Working these out, our chances of getting 0, 1 or 2 deaths with 2 animals are 0·25, 0·5 and 0·25 respectively. Our chances of getting 0, 1, 2, 3 or 4 deaths with 4 animals are 0·0625, 0·25, 0 375, 0·25 and 0·0625 respectively. The figures represent the relative frequencies of each of the 5 possible events expressed as a fraction of unity. That is, if the experiment of infecting four similar mice with a 50 per cent. killing dose were to be repeated N times, we should expect to get no deaths on 0·0625 N occasions, one death on 0·25 N occasions, and so on. In Fig. 237A these relative frequencies have been plotted vertically on a base line divided equally to represent the 5 possible events. We may calculate the frequencies in the same way for groups of any size, expanding the expression $(p + q)^n$, where n is the number of animals used. B and C represent the figures for $n = 8$ and $n = 12$. As n increases, the figure becomes less step-like, and shows a decreasing proportion of its total area at each end of the base line. We may note that the figures represent distributions of the relative frequencies of the various possible events. The first important fact about a frequency distribution is the average, or mean, frequency, which is used to characterize the distribution. In

A, B and C the mean frequency is in each case $n \times 0{\cdot}5$, *i.e.* 2, 4 and 6 deaths respectively. The other frequencies are symmetrically disposed on either side of the mean. (The symmetrical disposition is the result of taking $p = q$. If we take $p = 0{\cdot}3$, and therefore $q = 0{\cdot}7$, the relative frequencies of 4, 3, 2, 1 and 0 deaths in a batch of 4 mice are given by the binomial expansion of $(0{\cdot}3 + 0{\cdot}7)^4$, and are respectively 0·0081, 0·0756, 0·2646,

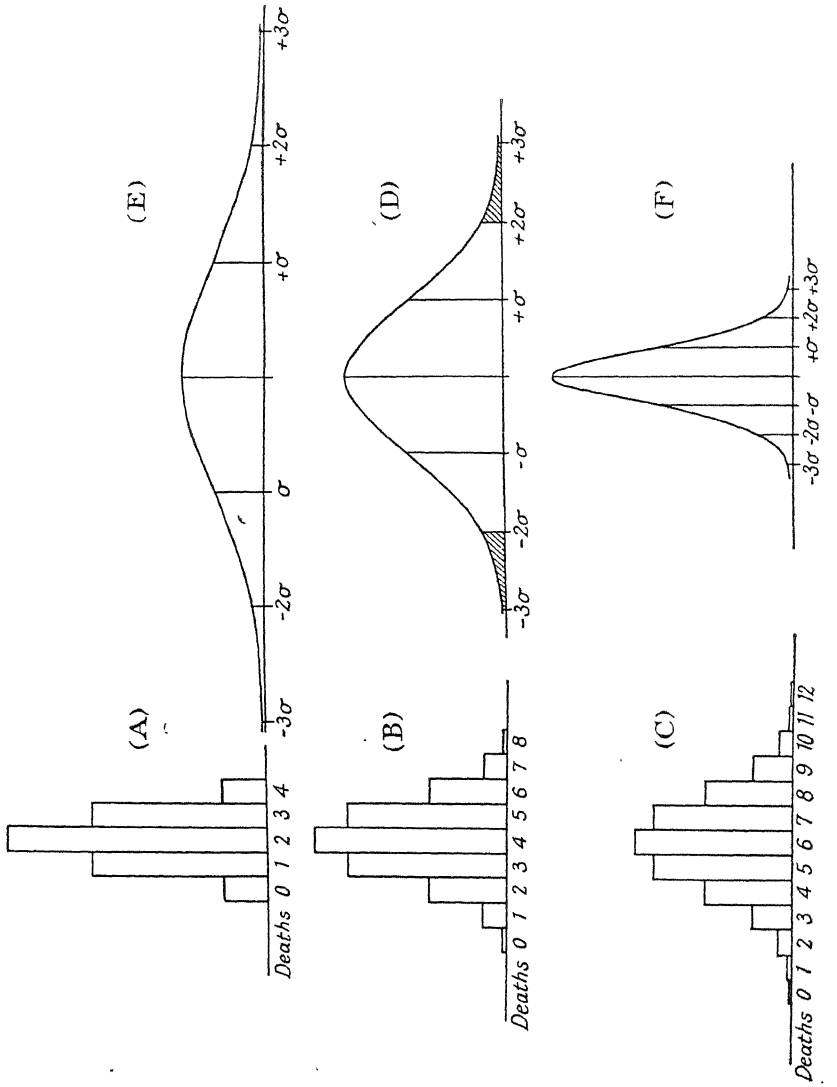


FIG. 237.

0·4116 and 0·2401. Here the distribution is not symmetrical; 1 death in four will occur with the highest frequency, and the mean death rate in 4 mice is 1·2).

Frequencies other than the mean frequency are conveniently recorded as *deviations* from the mean value. In A, for example, the death rates of 3/4 and 1/4 are regarded as deviations of + 1/4 and − 1/4 respectively from the mean death rate of 2/4; or,

expressed in percentages, as deviations of + 25 per cent. and − 25 per cent. from a mean of 50 per cent.

In experimental work we have often to compare death rates in several sets of animals differing in size and (see p. 997) in the resistance of the individual members. It is clear that the 3 groups in A, B and C are not fully comparable, even though all the animals are, by hypothesis, equally susceptible to the lethal agent. In A, for example, the frequency with which a deviation of + 50 per cent. from the mean will occur by chance is p^4, which is 0·0625 or about 6 times in 100 trials; whereas in C, the same deviation will occur by chance about twice in 10,000 trials, since p^{12} is 0·000244. More generally, as the relative heights of the columns in A and C show, the bigger deviations from the mean are less likely to occur in the test with the larger number of animals; and there is a greater probability of obtaining results near the expected mean death rate of 50 per cent. In comparing distributions, then, we must consider not only the mean, but also the range over which the deviations from the mean are spread; to do this we need a measure of the spread of deviations applicable to all distributions of this type. This measure we find in a value known as the standard deviation, which is the second important characteristic of a distribution.

TABLE 60

Deviation in Terms of the Standard Error; or Ratio between a Difference and its Standard Deviation.	Odds against this Deviation or Difference being the Result of Random Chance.
0·5	0·6 to 1
1·0	2·15 to 1
1·5	6·5 to 1
2·0	20·98 to 1
2·5	79·53 to 1
3·0	369·4 to 1
3·5	2,149 to 1
4·0	15,773 to 1
4·5	147,188 to 1

For the better appreciation of the standard deviation, we shall consider a distribution that is more general in form than A, B or C. These are step-like, being made up of a collection of parallelograms, the area of each of which corresponds to the frequency of each event. If we make n very large, say 1,000, and reduce the scale upon which we record the events, the figure for $(p + q)^{1000}$ approximates to a continuous curve of the form D. This curve is known as the *normal curve*. The distribution of frequencies of a great many biological measurements, such as the height of human beings of a certain age, or the resistance of a certain type of animal to doses of a certain poison, approximates to this mathematically defined curve. For reasons that will be found in the statistical text-books, we may assume with some confidence that in the measurements of resistance, etc., with which we are mainly concerned in immunity, the deviations from a mean value will be distributed much as they are in the normal curve, and that the arithmetic of the normal curve will be applicable to most of our data.

The standard deviation of a distribution that approximates to the normal may be obtained arithmetically, by squaring all the deviations from the mean, adding the squares together, dividing the result by the total number of observations, and taking the square root. The resulting figure, the "root mean square deviation," is usually written as σ. It has a positive and a negative value and is correspondingly measured to the right and left of the mean in graphic representation of the distribution. In D, for example, the base line has been marked off at $\pm\sigma$, $\pm 2\sigma$ and $\pm 3\sigma$, and verticals erected at these points. It will be seen that nearly all the events or observations summarized by the curve are included between the limits $\pm 3\sigma$. In fact, only some 0·027 per cent. of the observations lie outside $\pm 3\sigma$, and only about 4·5 per cent. outside $\pm 2\sigma$. Figures E and F, representing

normal curves in which the distribution of frequencies is respectively narrower and wider than that in D, are also divided in this manner, and it is obvious that the areas in D, E and F marked off by the verticals erected at multiples of the standard deviation in each case bear the same relation to the total area enclosed by the curve.

Now since the frequencies of *all* the events or observations are represented by the total area enclosed by the curve, we may express the chance of observing one of a given set of these events by the ratio of the area representing the set of events to the total area. Suppose we wish to know the chance of observing events whose frequencies deviate from the mean by more than $+ 2\sigma$. In Fig. 237 D it is clearly the ratio of the shaded area on the right of the vertical erected at $+ 2\sigma$, to the total area. This ratio is 1 : 43 96 ; or, as we shall find it more convenient to say, the odds against observing events of this nature are 42·96 to 1. The chance of observing a deviation greater than $+ 2\sigma$ and less than $- 2\sigma$ is in a similar way the ratio of the sum of the shaded areas at each end of the figure to the total area. Since the curve is symmetrical, the two shaded areas are equal, and the ratio is 2 : 43·96, or 1 : 21·98 ; that is, the odds are 20 98 to 1 against deviations greater than $\pm 2\sigma$. These odds have been calculated for all multiples and submultiples of σ in the normal curve and they are tabulated in Table 60 for values of σ from 0·5, 1·0, 1·5 . . . to 4·5. Beyond 4·5 the odds are so high that their exact value is of no interest.

In the particular cases with which we started, we may calculate the standard deviation as indicated above. There is a simpler method of calculation, however, applicable to these and any other distributions that are assumed to arise from the binomial expression $(p + q)^n$. The standard deviation is in fact equal to $\sqrt{npq}$. Applying this to distributions A, B and C in Fig. 237 we have

Distribution.	Mean.	Standard Deviation.
A. $(p + q)^4$	2 deaths	$\sqrt{1} = \pm 1$ death
B. $(p + q)^8$	4 deaths	$\sqrt{2} = \pm 1{\cdot}414$ deaths
C. $(p + q)^{12}$	6 deaths	$\sqrt{3} = \pm 1{\cdot}732$ deaths

Or if we regard mean and standard deviation as proportions, the latter becomes $\sqrt{\frac{pq}{n}}$ and the result is

Distribution.	Mean.	Standard Deviation.
A. $(p + q)^4$	0·5	$\pm$ 0·250
B. $(p + q)^8$	0·5	$\pm$ 0·177
C. $(p + q)^{12}$	0·5	$\pm$ 0·144
D. $(p + q)^{1000}$	0·5	$\pm$ 0·016

Suppose that we apply to 12 animals a test that will, on the average, kill 50 per cent. of them, what are the odds that we shall observe a mortality of 83·3 per cent. ? In this case $\sigma = \pm 1{\cdot}732$ deaths. An 83·3 per cent. mortality corresponds to 10 deaths, a deviation from the mean of + 4. This deviation differs from the mean by 4/1·732 times (= 2·31 times) the standard error. As we see from Table 60, the required odds lie between 21 to 1 and 80 to 1. In terms of proportions, the deviation is 0·83 − 0·5 = + 0·33 and this in terms of the standard deviation is 0·33/0·144, which as before is 2·31.

The applicability of the arithmetic of the normal distribution to our test animals depends on how our experimental data approximate to the curve.

In the kind of experiments with which we are usually concerned there are two factors that affect this degree of approximation. One is the number of animals we use. We shall never use enough to give us a really smooth curve. In fact our distribution will usually be nearer to that shown at C, Fig. 237, than to the true normal curve. The other factor is that while the normal curve is symmetrical, corresponding to the expansion of $(0{\cdot}5 + 0{\cdot}5)^n$, the curve with which we are actually concerned will usually be asymmetrical, corresponding, for example, to the expansion of $(0{\cdot}3 + 0{\cdot}7)^n$, for we shall not necessarily be dealing with a test that gives an average mortality of 50 per cent. But if the number

of animals in the group under test is not too small, say not less than twenty, and the chance of the less likely event, life or death whichever it may be, is not less than 0·2, the odds calculated on the basis of the standard deviation are sufficiently accurate.

It will, however, have been noted that we have, throughout the preceding discussion, assumed the possession of one very important piece of knowledge—the true average mortality involved in the procedure under test. Usually we do not know this. The question that faces us is far more often as follows : In a particular test we have observed a mortality of, let us say, 40 per cent. among a group of 30 animals. What are the odds, if we did exactly the same experiment over again, that we should, in this second test, observe a mortality of 60 per cent. or more ? The only assumption on which we can proceed in this case is that our observed 40 per cent. mortality is the true average mortality ; but it is by no means a safe assumption, and for that reason among others we shall be wise to suspect that the real odds are shorter than those we calculate.

So far we have been concerned with a single group of animals. But in most of our experiments we shall be comparing the reactions of two groups of animals treated in the same way except for the procedure under test, which is applied to the test group, the other being observed as a control. If the mortality rate in the two groups is different, we wish to know whether this difference might have arisen by chance in two groups that were in fact similar, or whether the odds against a chance effect are large enough to rule it out. If the groups are not different then the most likely event is a difference of 0. But the results from each group may by chance vary round the mean, and if one result happens to deviate largely in the positive direction and the other in the negative, the chance difference may be large. If we repeated the comparison many times, we should expect the larger chance differences to be infrequent, the smaller ones more frequent, and differences of 0 to be most frequent. Now it is found that *differences* between pairs of mean values derived from two normal distributions are themselves distributed normally ; and this distribution of *differences* has a standard deviation, or as it is usually called, a standard error. This standard error is easily obtained, since it is the square root of the sum of the squares of the standard errors of the two means from which the difference was derived. In our simple binomial examples, where x_1 and x_2 are the numbers of animals dying in the two groups, the standard error of a mean proportional death-rate x_1/n_1 found in a group of n_1 animals in which the expectation of death is p_1 is $\sqrt{p_1q_1/n_1}$, and similarly of x_2/n_2 is $\sqrt{p_2q_2/n_2}$. The standard error of the difference $x_2/n_2 - x_1/n_1 = d$, is then

$$\sqrt{p_1q_1/n_1 + p_2q_2/n_2}.$$

Now if the difference has arisen by chance, *i.e.*, the two groups do not differ in any significant way, the best measure we have of the real chance of death is the death rate in the 2 groups as a whole. This, the mean chance of death, is $(x_1 + x_2)/(n_1 + n_2)$, which we may call p_0. The mean chance of survival, $q_0 = (1 - p_0)$. The standard error, then, of the mean x_1/n_1 is now $\sqrt{p_0q_0/n_1}$ and that of x_2/n_2 is $\sqrt{p_0q_0/n_2}$; and the standard error of the difference is $\sqrt{p_0q_0\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$ or if we are dealing in percentages

$$100\sqrt{p_0q_0\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

Let us now take a concrete example. Thirty mice that had been repeatedly inoculated with a killed suspension of *Salm. typhi-murium* and 30 uninoculated mice to serve as controls were injected intraperitoneally with a constant dose (1,000 bacilli) of living *Salm. typhi-murium*. They were then observed over a period of 28 days. During this time 16 of the 30 vaccinated mice died of the infection, and 29 of the 30 controls. The survivors were killed on the 28th day and cultures were taken from their spleens. *Salm. typhi-murium* was recovered from 10 of the

14 surviving vaccinated mice, but not from the one surviving control. We may tabulate these results as follows:

Number of mice surviving on 28th day:

Vaccinated	14 = 46·67	per cent.
Controls	1 = 3·33	,, ,,
Difference	13 = 43·34	,, ,,

Number of mice showing no evidence of infection (completely resistant):

Vaccinated	4 = 13·33	per cent.
Controls	1 = 3·33	,, ,,
Difference	3 = 10·00	,, ,,

The vaccinated mice show an advantage over the controls of 43·34 per cent. in regard to survival rate, but of only 10 per cent. in regard to their ability to resist completely the injection of this number of organisms. What are the odds against each of these observed differences in behaviour having been due to chance?

Of 30 vaccinated mice 14 survived: of 30 control mice 1 survived.

$$q_0 = \frac{14+1}{30+30} = \frac{15}{60} = 0{\cdot}25$$

$$p_0 = 1 - q_0 = 0{\cdot}75$$

$$\sqrt{p_0 q_0 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)} = \sqrt{0{\cdot}75 \times 0{\cdot}25 \times \left(\frac{1}{30} + \frac{1}{30}\right)}$$

$$= \sqrt{\frac{0{\cdot}75 \times 0{\cdot}25}{15}} = 0{\cdot}1118$$

The standard error then of differences that might arise by chance expressed as a percentage is $\pm$ 11·18, and the distribution of the possible chance differences round an expected mean difference of 0 per cent. may be represented as in Fig. 238.

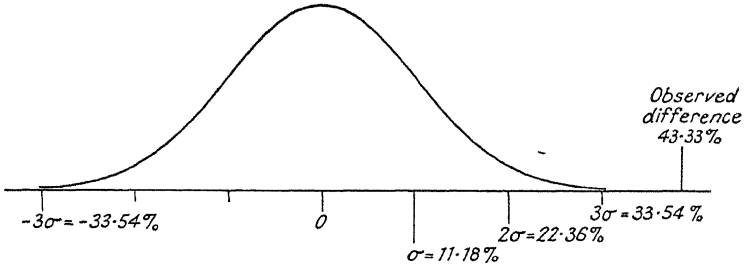


FIG. 238.—Showing the hypothetical frequency distribution of the chance differences between means of two samples derived from the same population.

Since chance differences may be either positive or negative, the observed difference must be considered as $\pm$ 43·34. The position of + 43·34 is recorded in the figure; that of — 43·34 will clearly lie a similar distance left of the O value. In both cases the difference is 3·87 times the standard error. By reference to Table 60 we find the odds against observing such a difference as this as the result of chance lie between 2,149 to 1 and 15,773 to 1; they are in fact 9,190 to 1. These are very long odds, so that we shall conclude with considerable confidence that we have

increased the ability of our mice to withstand the test dose of bacteria, by our preliminary injections of killed bacteria.

Have we increased their ability to resist the test dose completely ?

Of the 30 vaccinated mice, 4 proved completely resistant; of the 30 control mice 1 proved completely resistant.

Taking q to express resistance—

$$q_0 = \frac{4+1}{30+30} = \frac{5}{60} = 0{\cdot}0833$$

$$p_0 = 1 - q_0 = 0{\cdot}9167$$

$$\sqrt{p_0 q_0 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)} = \sqrt{\frac{0{\cdot}0833 \times 0{\cdot}9167}{15}} = 0{\cdot}07136.$$

The observed difference in the proportion of mice completely resistant is 10·00 per cent., the standard error expressed as a percentage is 7·136. The observed difference is 10/7·136 (1·4) times its standard error.

Turning to Table 60 we find that the odds against observing such a difference as this as the result of chance are about 5 to 1. These are very short odds; and we should clearly place no confidence in them. Taking our observations as a whole we should conclude that, by the method of immunization employed, we had almost certainly increased the ability of our mice to survive the test dose of living bacteria, but that the effect on their ability to resist infection entirely was relatively slight, and quite possibly insignificant.

This is one selected example. It may be useful to note how the odds vary according to the number of deaths or survivals observed in two groups, each of 20 animals, one group being treated by some procedure under test, the other serving as untreated controls. Both groups, of course, will be subjected to the same dose of living bacteria, toxin or other agent against which the treatment is directed. These odds are shown in Table 61, very short odds being neglected and odds of over 1,000 to 1 being no further specified. No odds are shown for less than 5 deaths in one group or more than 15 deaths in the other group. Where the number of animals in each group is as small as 20, and the number of deaths in each group is very small (say 4 or less) or very large (say 16 or more) the assumptions on which these calculations are based no longer hold. The example of the fully resistant mice among our groups of 30 was not above criticism in this respect.

In all the examples discussed above we have assumed that, so far as chance has entered into our observations at all, it has been in the form known to the statistician as " the error of random sampling " ; but there are other kinds of " chance," using that term in a broader and less technical sense. To take a crude example, suppose that we were testing a method of immunizing rabbits against a particular toxin, and failed to allow for the fact that different breeds of rabbit may differ significantly in their resistance to any agent of this kind. We might include in our " treated " group a majority of animals of the more resistant breed, and in our control group a majority of animals of the more susceptible breed. Under these conditions our " treated " group would probably show a statistically significant advantage, even if our treatment had, in reality, been quite without effect. " Chance " would be a charitable term for this particular piece of experimental carelessness ; but other factors, not nearly so obvious as differences in breed, or age, or sex, may influence the reaction of our experimental animals to the test

we are employing. An essential part of a good experimental technique is, as Fisher emphasizes, that all possible means shall be used to "randomize" the conditions of test, *i.e.* to reduce the unknown variables to influences of the same type as those that determine whether a tossed coin shall come down "heads" or "tails."

It is of interest to inquire how far we can do this in practice. Not many workers have taken the trouble to test their technique in this way, but Lockhart (1926) records observations bearing directly on the point at issue. In each of four experiments, several groups of 20 mice were injected with the same dose of the same culture of a pathogenic organism (*Salm. typhi-murium*) and the number dying in each group during the succeeding 14 days was noted. In one experiment 9 groups were injected; the deaths were 16, 16, 17, 19, 18, 17, 15, 17 and 14. This gives 36 differences between pairs. The difference between 14 and 19 deaths, the largest observed in this group, should, as will be seen from Table 61, occur on the average once in 27 comparisons. In a second series of 5 tests the deaths were 18, 17, 13, 16 and 13; this gives 10 differences between pairs. The difference between 13 and 18 deaths would be expected to occur, on the average, once in 17 comparisons.

In a third series of 5 groups the deaths were 5, 2, 4, 3 and 5; there is here no difference that would not be expected on our assumption, but the death rate is very low in all groups. In a fourth series of 5 groups the deaths were 19, 20, 19, 20 and 19; there is no unexpected difference, but the death rate was very high in all groups. Such a series of tests may be regarded as a trial of the adequacy of our experimental methods. If the distribution of deaths actually observed in different groups that have, within the limits of experimental control, been subjected to exactly the same treatment agrees closely with that expected on the assumption that the only differences that occur will be those due to the error of random sampling, then our technique may be regarded as highly satisfactory. If our observed distribution differs widely from that expected, then we have not succeeded in eliminating other disturbing factors, of unknown nature, which may mislead us when we come to apply a known procedure to one group in order to test its effect.

Lockhart's figures suggest that he was reasonably successful in randomizing his experimental conditions. It is, however, doubtful whether equally satisfactory results can always be attained in immunological tests of this kind, and it will perhaps be wise to make a practice of demanding rather long *calculated* odds before accepting an observed difference in behaviour as statistically significant. We might, for instance, fix an arbitrary limit for the ratio of a difference to its standard error at 3 : 1, which would give us calculated odds of 369 to 1 against the observed difference being due to chance. These odds, may, of course, be too high, and might lead us to ignore differences that are possibly significant. But if we adopt lower odds we may attach significance to differences that are in fact meaningless. In dealing with observations from the ward, or from the field, where fresh data cannot quickly or easily be collected, it is desirable that attention should be drawn to any points of possible interest or importance, with due emphasis where necessary on the allowance that must be made for sampling errors. In experimental work the case is rather different. The investigator will clearly be wise to take any hint that is offered by his quantitative results; but if he desires to publish his findings and draw conclusions from them, it is reasonable to insist on a stricter standard. There is nothing to prevent him from repeating any experiment that has given a statistically dubious answer; and this is clearly what he should do.

Experiments should, indeed, always be repeated, even when a single trial has given a result that passes all the conventional tests of statistical significance. The

TABLE 61

SHOWING (IN THE FORM $x:1$, x ALONE BEING GIVEN) THE ODDS AGAINST DIFFERENT COMBINATIONS OF DEATHS IN TWO GROUPS, EACH OF 20 ANIMALS, ON THE ASSUMPTION THAT THE DIFFERENCE BETWEEN THE TWO GROUPS IS DUE TO ERRORS OF RANDOM SAMPLING.

Number of Deaths in First Group.	Number of Deaths in Second Group.															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
5	58	12	—	—	—	—	—	—	—	—	9	18	39	89	228	633
6	127	26	8	—	—	—	—	—	—	—	—	8	17	37	87	228
7	276	55	16	6	—	—	—	—	—	—	—	—	8	17	37	89
8	633	122	34	12	—	—	—	—	—	—	—	—	—	8	17	39
9	>1000	285	75	25	10	—	—	—	—	—	—	—	—	—	8	18
10	,,	702	172	54	20	9	—	—	—	—	—	—	—	—	—	9
11	,,	>1000	422	122	44	18	8	—	—	—	—	—	—	—	—	—
12	,,	,,	>1000	304	100	39	17	8	—	—	—	—	—	—	—	—
13	,,	,,	,,	807	250	89	37	17	8	—	—	—	—	—	—	—
14	,,	,,	,,	>1000	678	228	87	37	17	8	—	—	—	—	—	—
15	,,	,,	,,	,,	>1000	633	228	89	39	18	9	—	—	—	—	—
16	,,	,,	,,	,,	,,	>1000	678	250	100	44	20	10	—	—	—	—
17	,,	,,	,,	,,	,,	,,	>1000	807	304	122	54	25	12	6	—	—
18	,,	,,	,,	,,	,,	,,	,,	>1000	>1000	422	172	75	34	16	8	—
19	,	,,	,,	,,	,,	,,	,,	,,	,,	>1000	702	285	122	55	26	12
20	,	,,	,,	,,	,,	,,	,,	,,	,,	,,	>1000	>1000	633	276	127	58

single trial cannot, except by analogy, tell us anything about happenings outside its own particular conditions; and we can never be quite sure that these conditions do not include some determining factor other than that, the effect of which we are trying to investigate. By repeating an experiment several times, we are testing, in the only way we can, for the intrusion of unknown factors other than errors of random sampling. If each of several tests gives us a statistically significant answer, and each answer points in the same direction, we shall have some justification for stating our conclusion in general as opposed to particular terms; but if some tests give different answers from others we must solve the problem of why these differences occur before we can accept the indication given by the original experiment.

The question thus arises, assuming a limit to the number of experimental animals that we have available, how we can use them to the best advantage in solving any particular problem. Shall we carry out one, or two, experiments with large numbers of animals in our test and control groups? or, shall we make several experiments, each on a smaller scale?

The answer would seem to be that each test should be made on a number of animals sufficiently large to enable us to detect a difference of the order we are expecting, making allowance for errors of random sampling. When this point has been reached we shall gain more by repeating the experiment, using groups of a similar size, than by doing one very large experiment, in which the effect of sampling errors would be considerably reduced, but which would give us no information in regard to other intrusive factors.

In most laboratory experiments, in which we are expecting a high mortality, usually approaching 100 per cent., in our control group, and are hoping for a much lower mortality, say 30–60 per cent., in our treated group, we shall gain more information by repeating our experiment six times, using 25 animals in each group, than by doing one experiment with 150 animals in each group. The best procedure, in any given case, will depend on the size of the difference we are expecting. Sometimes, particularly in the application of a therapeutic measure in human disease, quite a small difference in mortality is an objective well worth attaining; and, if we wish to determine a difference of this size, our groups in any individual trial must be relatively large.

Let us take a concrete example. Certain laboratory experiments have led to the production of a serum which, on *a priori* grounds, may be expected to have some curative influence on a particular infective disease. Like all new curative measures it *must* be subjected to a period of trial before it is adopted for routine use. Our expectations may not be realized. The serum may be quite useless in practice. It is not a question of trial or no trial, but of a well-planned trial that will give us a definite answer as quickly as possible, as against a planless, haphazard series of trials that will take a long while to tell us very little, perhaps at a considerable expense in lives. Let us suppose that the real average effect of the serum is to reduce the case mortality of the disease from 30 per cent. to 20 per cent. —not, perhaps, a dramatic result but one that will save a large number of patients if the disease is a prevalent one. The only way in which the existence of such an effect can be quickly and certainly established is to test the serum on an adequate group of cases and compare the mortality in this treated group with the mortality in an untreated group. The two groups must if possible be compared over the same period of time and in the same place, since the case mortality of almost all

acute infective diseases fluctuates from time to time and from one locality to another. They must also be similar in all relevant respects other than the administration of the serum; for example, they must all be of the same race, and the age and sex distribution within the two groups must be approximately the same. Let us see what will happen if such a trial is carried out on two groups, each containing 10, 50, 100, 150, 200, 250 or 500 patients. The relevant figures are set out in Table 62, the standard error being calculated on the percentage basis.

TABLE 62

SHOWING THE RATIO [DIFFERENCE IN MORTALITY/S.E.] FOR TREATED AND UNTREATED GROUPS IN THE CASE OF AN ANTISERUM WHICH PRODUCES A DECREASE IN CASE MORTALITY FROM 30 PER CENT. TO 20 PER CENT.

Number in Each Group.	Deaths in Treated Group.	Deaths in Untreated Group.	Difference. per cent.	S E.	Difference in Mortality — S.E.
10	2	3	10	19 365	0·52
50	10	15	10	8 660	1·15
100	20	30	10	6·124	1·63
150	30	45	10	5 000	2 00
200	40	60	10	4·330	2·31
250	50	75	10	3·873	2·58
500	100	150	10	2·739	3·65

Quite clearly a trial with 10 treated and 10 untreated patients is useless. In different trials on this scale, one observer will record more deaths in the treated group, another in the untreated. There will be hopeless confusion. The position with groups of 50 treated and 50 untreated patients is not much better. In about one of every four trials a difference such as this would be observed as the result of chance alone. There will be sharp differences of opinion as to whether the treatment does *any* good, and no possibility of assessing even approximately how much good it does. As the number of patients in each group increases the significance of the result recorded also increases—not proportionately to the number itself but in proportion to its square root. After a trial on two groups each of 100 patients we should begin to think it probable that the serum had done some good. After a trial on two groups each of 200 patients we should be inclined to adopt this view as a provisional conclusion pending further evidence. If trials on this scale were repeated we should be surprised if there were many unfavourable reports. After a trial on two groups of 500 patients we should have no reasonable doubt that our serum had reduced the case mortality. Moreover, with groups of this size we should begin to think that the benefit to be expected from our serum was appropriately represented by a decrease in case mortality from about 30 to about 20 per cent.—that is, by a saving of one patient in three who would otherwise have died—since the chance of the observed decrease differing from the real average by any given amount grows less and less as the number in the group increases.

It is of very real importance that trials of any new therapeutic measure should be planned and carried out on an adequate scale. Otherwise the period of so-called trial may be extended far beyond the necessary limits; and a useless measure may be employed sporadically for years, or—what is far more serious—a useful

measure may fail to obtain general recognition because the reports on inadequate trials are contradictory and confusing. Any student who cares to master the history of the use of anti-pneumococcal serum (see pp. 1675-1679) will find a case in point.

It should be noted that, in the case of a proposed method of prophylaxis, our field trials will often have to be carried out on a far larger scale, because the risk of contracting a particular infective disease is often far smaller than the risk of dying from it when contracted. If the attack rate of a disease is very low, our immunized and non-immunized groups must be greatly expanded—often to many thousands of persons—before the number of cases in our control group will be large enough for us to establish the significance of any difference in morbidity that may be noted.

If, however, we can select special groups that are for some reason exposed to a particularly high risk of infection—such for instance as the nurses or attendants in a fever hospital—we may be able to detect the effect of a given prophylactic method by the immunization of relatively small groups (see p. 1474). The same will hold, of course, for the population at large in the case of infections with a very high attack rate, such as the common cold (see p. 1654).

We have so far been concerned with problems in which each animal, or each patient, gives a qualitative result—death or survival, infection or no infection. There is obviously another kind of problem in which each animal may give a quantitative result: it may live so many days, or lose so much weight, or its temperature may rise so many degrees, and so on. We deal with a problem of this kind by a method that is essentially similar to that described above. We have, say, a number of values for the time to death in each group, and from these two distributions of values we calculate the mean time to death and the standard deviation of the distribution. We compare the two means as before by finding the difference between them, and finding the value of the difference in terms of its standard error. As before, we need the standard error of each mean. The general expression for this quantity is

$$\text{Standard error (S.E.) of Mean} = \text{S.D.}/\sqrt{N}$$

where S.D. is the standard deviation, and N the number of observations. In the particular case, N is the number of measurements of mean time to death. (The formula $\sqrt{p_1q_1/n_1}$, employed on p. 987 for the standard error of the mean was valid only for a simple binomial distribution when $N = 1$.)

The ratio of the difference to its standard error gives us the value we need in order to obtain, from Table 60, the odds against the observed difference having been due to chance.

To take a concrete example, omitting the necessary calculations.

A group of 30 mice that had received certain immunizing injections, and 30 untreated mice as a control, were injected intraperitoneally with 1,000 *Salm. typhi-murium*. During the subsequent month 25 of the immunized mice died, and 28 of the controls. This was a trivial difference. But the mean time to death of the 25 immunized mice was 10·52 days with a standard error of 0·960 days, while the mean time to death of the 28 control mice was 5·93 days with a standard error of 0·924 days. The difference is 4·59 days, and the standard error of the difference is $\sqrt{0{\cdot}960^2 + 0{\cdot}924^2} = 1{\cdot}33$. The ratio of the difference to its standard error is $4{\cdot}59/1{\cdot}33 = 3{\cdot}45$, and the odds against observing such a difference as the result of chance (Table 60) are about 2,000 to 1. The conclusion is that we have almost certainly made the disease less acutely fatal as the result of an immunization, although we have not increased the resistance of our mice to the point at which their chance of survival is significantly increased.

THE MEASUREMENT, OR COMPARISON, OF TOXICITY OR VIRULENCE

We often want to determine the minimal lethal dose of a bacterial toxin, or of a living bacterial culture. Still more often we want to compare the minimal lethal dose of two toxins, or of two cultures. Clearly we must first titrate graded doses of the two cultures against a number of groups of animals. The results of such titrations are instructive. If we divide a large number of similar animals into groups of equal size, and administer to each group one of a series of increasing doses of a lethal culture or toxin, we may observe, if the scale of doses was suitably chosen, that none of the animals dies in the group receiving the smallest dose. With each increase of dose a larger death rate is observed and in the group receiving the largest dose all the animals die.

TABLE 63

Dose a in Arbitrary Units.	Percentage Mortality in Groups of 100 Animals.	Increase in Percentage Mortality with each Increment of Dose.
2	0	—
3	1	1
4	4	3
5	10	6
6	22	12
7	40	18
8	60	20
9	78	18
10	90	12
11	96	6
12	99	3
13	100	1

Table 63 shows a set of hypothetical percentage mortalities in 12 groups of 100 animals, each group being given one of a series of doses of a lethal toxin. Each dose differs by one unit of measure from the preceding dose. If we plot death rate against dose, as in Fig. 239(A), we obtain a *characteristic* curve (Trevan 1927, 1929, 1930) for the toxin, or, more specifically, a dose-response curve. The third column of Table 63 records the values obtained by subtracting from the mortality rate for a given dose of a units, the mortality for the preceding dose, $(a - 1)$ units. Inspection both of column 3 and Fig. 239(A) reveals one of the most important features of the dose-response curve, namely that the rate of change of percentage mortality is greatest in the middle range of the curve. At the extremes, a relatively large change in dose results in a small change in mortality. That is, for a certain grading of doses, we shall be able to determine doses that kill 50 per cent. of a group of animals with much greater accuracy than those that kill 100 per cent. Trevan introduced a convenient notation for these various doses. The 50 per cent. killing dose is symbolized by LD50, where LD = lethal dose; the 100 per cent. killing dose by LD100, and so forth. If, as in Fig. 239(B), the dose-response curve is steep, the inaccuracy of finding the exact LD100 is clearly less than in (A). In the past, with toxins like that of *C. diphtheriæ*, which have a steep dose-response curve, the LD100 has successfully been used as a measure

of toxicity. It corresponds, in fact, to the minimal lethal dose (p. 238). With other toxins, and with cultures of pathogenic bacteria, the dose-response curve is far less steep. In Table 65, for example, the dose of the Ellinger strain of bacteria increases by 100-fold at each step, yet the rate of change of percentage mortality is relatively small, especially as the LD100 is approached. The LD50 can be more

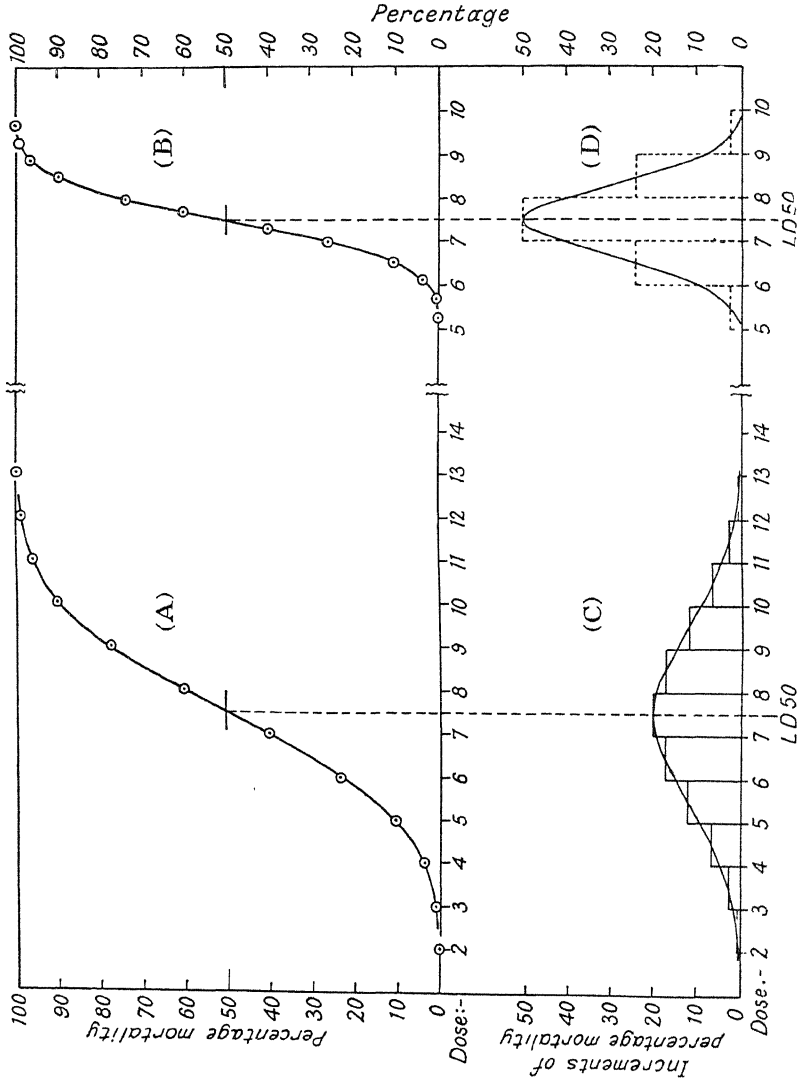


Fig. 239.

accurately measured, and even with steep dose-response curves, it is to be preferred to the LD100. In essentials, then, toxicity or virulence is best measured in terms of the LD50.

It is instructive to inquire why the dose-response curve has this particular S-shape. If we assume that each group of 100 animals is representative of the total of 1,200 animals

from which they were drawn, it is clear that the figures in column 3 of Table 63 represent the proportion of all the animals which are susceptible to a dose of a units, but not to a dose of $(a - 1)$ units. In each case, then, we may say that the recorded proportion of animals is on the average susceptible to a dose half-way between these two values, that is, $(a - 0{\cdot}5)$ units. Thus, the 3 per cent. of the animals susceptible to 4 units but not to 3 units, are on the average susceptible to 3 5 units, and so forth. Plotting these proportions against doses as abscissæ we obtain the curve in Fig. 239 (C), which is in effect a frequency distribution of susceptibilities in the population of animals employed. The average, or mean resistance, corresponds to the LD50. The hypothetical curve B represents the response of another batch of animals to the toxin, estimated from a series of doses increasing by 0·4 units. The curve (D), the distribution of susceptibilities derived from it, has been drawn on a scale of unit increases in dose, for comparison with the curve (C). It is clear that the steeper dose-response curve corresponds to a narrow distribution of susceptibilities obtained in the performance of the test. These distributions. it should be noted, are distinct from the distributions of chance effects discussed on p. 983, which were derived from an ideal population of identical animals infected with a fixed dose. Here we are estimating how widely the population of animals varies in resistance to graded doses, though each mortality measurement is subject to the error discussed above. In estimating the mortalities from which the dose-response curve is built, we shall have to take into account both sources of error—the error due to heterogeneity of the animal population tested, and the error due to chance effects in small groups of animals. If the death-rate of a group is known, the last can be estimated by the $\sqrt{npq}$ formula; the first, only by measuring the slope of the dose-response curve.

It will be clear that the more homogeneous the animals used, the more they approximate to an ideal test reagent and the smaller the error of measurement with a group of animals of a given size. Some breeds of laboratory animals used for biological assay of this kind are grossly heterogeneous, but it is possible to reduce the heterogeneity by inbreeding of selected strains. These inbred strains, which may or may not be pure lines, often yield much steeper dose-response curves than those of the parent strains, and their use in biological assay permits either a greater increase in the accuracy of an assay for the expenditure of the same number of animals, or measurements of the same accuracy with smaller numbers of animals. For example, Prigge (1937) records that the ratio of the smallest to the largest dose of diphtheria toxoid required to immunize the individual animals in a mixed laboratory strain of guinea-pigs against a standard injection of toxin was 1 : 32,000. Similar tests of pure line strains inbred by brother-sister matings revealed a strain for which the ratio was as small as 1 : 25.

The LD50 may be estimated directly from a dose-response curve. One simple method is to fit a straight line to observed mortalities whose values lie between 25 per cent. and 75 per cent. and read off the LD50. Its accuracy depends on the assumption that the curve is approximately a straight line between these limits. It is limited in that observed mortalities greater than 75 per cent. and less than 25 per cent. have no weight in determining the final answer. Accurate and relatively simple methods of estimating the LD50 from *all* the observed mortalities are available. The technical and mathematical considerations (of which we have discussed only very limited aspects) involved in assessing the significance of the necessary *in vivo* titrations are beyond the scope of this book. Those who desire further information should consult the papers by Gaddum (1933), Irwin (1937), Bliss (1938), and Prigge and Schäfer (1939). Reed and Muench (1938) and Irwin and Cheeseman (1939*a*, *b*) have developed simple arithmetical methods for use with small-scale laboratory data.

As we have noted above, the killing power of exotoxins and other non-living substances is assayed with comparative ease. Two concrete examples will serve to illustrate the difficulties with which we are faced when we endeavour to determine the average lethal dose of a bacterial culture, or to compare the virulence of two bacterial strains.

A series of six dilutions were prepared from a culture of pneumococcus of moderate

virulence, and the same amount of each dilution was inoculated into 40 mice. The doses administered, in terms of living pneumococci, were 10^6, 10^5, 10^4, 10^3, 10^2 and 10. Two of the mice that received each dose were then allotted at random to each of 20 numbered groups. Each group thus consisted of 12 mice, 2 for each dose tested. The deaths are recorded in Table 64.

TABLE 64

SHOWING THE NUMBER OF DEATHS IN 20 SAMPLES, EACH OF 12 MICE, INOCULATED WITH THE SAME SERIES OF GRADED DOSES OF THE SAME CULTURE OF PNEUMOCOCCUS.

Dose	Group.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
10^6	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
10^5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
10^4	1	2	1	1	2	1	2	1	1	2	2	2	1	2	1	0	1	0	2	1
10^3	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
10^2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Adding across the rows, the percentage mortality for the whole series was, with the falling doses, 100, 97·5, 65·0, 10·0, 0 and 0. The average lethal dose was probably something between 10,000 and 1,000 pneumococci. But if we had used two mice for each dose—and it would have been a highly conventional procedure—we should have said in the case of groups 10 and 11 that the average lethal dose was about 1,000 pneumococci; in the case of groups 1, 2, 5, 6, 7, 12, 14 and 19 that it lay somewhere between 1,000 and 10,000 pneumococci; in the case of groups 3, 4, 8, 9, 13, 15, 16, 17 and 20 that it was about 10,000 pneumococci; and in the case of group 18 that it was about 100,000 pneumococci. Had we been comparing 20 different strains of pneumococci, we might have been unwise enough to hazard the statement that strains 10 and 11 were about 100 times more virulent than strain 18; whereas, as this experiment showed, there would have been no adequate reason to assume any difference in virulence.

As an illustration of the comparison of the virulence of two different strains of a given bacterium we may take the data recorded by Lockhart (1926) in regard to the mortality produced by different doses of two strains of *Salm. typhi-murium*, "Ellinger" and "A52," all injections being given intraperitoneally. The figures are set out in Table 65.

TABLE 65

Dose.	Number of Mice in Each Group.	Mortality per cent. Strain A52.	Mortality per cent. Strain Ellinger.
10,000,000	25	92	96
100,000	25	48	88
1,000	25	44	56
10	25	16	24

We can feel fairly confident that the Ellinger strain is more virulent than the A52 strain; at each dose level it has given a higher mortality. But we clearly cannot express our result as any numerical ratio. If this is the best we can do with 200 mice, we must be satisfied to detect gross differences in virulence, so far as this organism is concerned.

THE STANDARDIZATION OF IMMUNOLOGICAL PRODUCTS

It may be noted here that the method of direct comparison is always employed when we wish to standardize an immunological reagent, *i.e.* to express its value in terms of some arbitrary unit. Thus if we wish to standardize an anti-diphtheria serum in terms of units of antitoxin, we compare its protective value with that of a particular antitoxic serum which is accepted by international agreement as the standard of reference. This standard serum has been submitted to very careful assay and has been allotted an agreed but arbitrary value in terms of antitoxin units. Once this value has been allotted it remains the permanent standard for the antitoxin, or other reagent, in question.

The statistical considerations involved in these comparative neutralization tests are of the same kind as those involved in comparisons of toxicity or of virulence; but the size of the sampling errors, and hence the accuracy of our titrations, are modified by the actual procedure adopted in each particular case. Here again reference may be made to papers quoted in the preceding section.

A NOTE ON THE RECORDING OF QUANTITATIVE RESULTS

It is a common habit among immunological workers to record their quantitative results in the form of curves or charts. The advantage is obvious. The meaning leaps to the eye. The danger is that it sometimes leaps before one looks It is a very safe rule never to trust the tale told by a curve until the quantitative data on which it is based have been critically examined. If the points through which a curve is drawn are based on figures which are subject to errors of random sampling, and if the data permit the standard deviation to be calculated, it is a good plan to mark off three times the standard deviation above and below each point on the curve, join up these points, and, realizing that the curve might run anywhere within the broad band thus demarcated, reconsider its actual significance.

Tables are duller things than curves, and rather more trouble to master. But they tell much more illuminating tales. They should always state quite plainly the number of observations made, never percentages without this information. If a table has a column headed " per cent." always look first at the columns giving the figures on which the percentages are based. Occasionally it will be found that 33·33 per cent. in the last column corresponds to a 1 and a 3 in the columns preceding it. In such cases, of course, the 33·33 per cent. is quite meaningless.

THE VALUE OF QUANTITATIVE MEASURES OF ERROR

The quantitative results of biological tests or observations can be used with confidence only if the error to which the estimation is subject is known with sufficient accuracy. Statistical analysis of the experimental data may reveal both the errors due to the variability of the material used, and the errors inherent in the technique of experiment; and with a measure of the total error we may assess the statistical significance of any numerical result. Without such an estimate of error, we can compare neither two experiments by one worker, nor the experiments of several workers. It is not, however, enough to submit the finished data to statistical tests. Simple statistical principles of the kind we have been considering are not rules to be applied in drawing conclusions from an experi-

ment that has been done—*they are part of the experiment itself, an essential factor in its proper planning.*

The statistical method does not consist merely in applying arithmetical analysis to the numerical results of an experiment. It includes the detection and elimination of bias, and the proper appreciation of the variables to be considered, both in the design and technique of the experiment, and in its analysis. It is often possible, for example, by a simple consideration of the numbers of animals available for a given test, to say that the experiment is unlikely to give a significant answer. In such a case, the test is much better made upon a larger scale, or abandoned altogether.

A word of warning is needed. No conviction of the necessity for the proper design of experiments, and for the statistical analysis of the results obtained, should lead us to over-estimate the support that a statistically significant result can give to the particular hypothesis the experiment was intended to test. As Fisher (1942) points out, all that a properly designed experiment can test is the "null hypothesis"; that is, the hypothesis that there is no difference between the control and test systems that we are studying. If, for example, the calculated odds prove to be 3 to 1 against a chance effect in the defined conditions of a therapeutic test of a fluid A in a fatal bacterial infection, no one could decide, without further experiment, whether the effect observed was due to "chance" or to A. If the odds prove to be 1,000 to 1, we can say that the effect is unlikely to be an example of a chance variation of the kind we expect from the materials used in the test; but we cannot say that it was *therefore* due to the administration of a few millilitres of the fluid A. The statistically significant result indicates that the test and control groups differ in some important respect and therefore permits us to consider the fluid A as a possible cause of that difference; but the decision that A *is* the cause of the observed reduction in mortality in the test group is independent of any statistical demonstration of significance and in a final analysis depends on the experimenter's knowledge of his particular science and his appreciation of the consistency of his ideas of A's action with the general theory of that science. If A was in fact water, the conclusion, no matter how statistically significant the experimental result, that a small quantity of water had cured the infection, would rightly be considered as suspect. If A, on the other hand, was a solution of a substance known to be antibacterial and non-toxic to the animals used in the experiment, then these pieces of scientific knowledge, coupled with the disproof of the null hypothesis, would go far towards justifying the conclusion that A was capable of curing a particular type of infection.

The aim, then, of the statistical method in experiments involving the comparison of two or more groups of events is to discover how far the null hypothesis is likely to be true; and only in those cases where the null hypothesis is disproved is there any justification for considering the possibility that an observed effect resulted from any of the deliberate variations in the conditions of the experiment.

The proper design and analysis of quantitative tests is essential for the efficient practice of a science. That the record of immunological work in the past contains few examples of statistical analysis is no proof that such analysis is unnecessary. Sometimes the results of an experiment are so clear cut that any arithmetical analysis is unnecessary; on many other occasions it will be found that the data are incapable of supporting the conclusions reached by the investigators. There is no doubt that much of value has been learned without any consideration of

statistical design, but it is justifiable to suppose that the knowledge could often have been achieved with a great deal less expenditure of time and materials had the experiments been designed more efficiently.

The voyager who ignores tidal streams and compass deviations will arrive somewhere in the long run; but he is less likely to identify correctly the place at which he has arrived than is another who allows for these variables by the accepted methods of elementary navigation.

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CHAPTER 44

THE MECHANISMS OF BACTERIAL INFECTION

BEFORE we can discuss the mechanisms by which the host resists infection, we need as clear a picture as possible of the ways in which the parasite injures the host. Some parts of the picture are confused, others sketchy; but parts of the main outline are quite clear and definite, and it is with these that we shall be mainly concerned.

Specific Infection. Koch's Postulates.

Although not immediately pertinent to our main problem, a brief discussion of the kind of evidence which will justify the conclusion that a particular disease is caused by a particular parasite forms a convenient introduction to the consideration of infective processes in general, and raises points of some interest and importance.

A series of conditions, which must be fulfilled in order to establish such a causal relationship, are set out in most bacteriological textbooks under the title of "Koch's Postulates," though there is no clear evidence that Koch ever enunciated them in the categorical form in which they are usually quoted. They run in general terms as follows:

(1) The organism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed.

(2) The organism should be cultivated outside the body of the host, in pure culture, for several generations.

(3) The organism so isolated should reproduce the disease in other susceptible animals.

We should to-day recognize additional ancillary criteria which help us to form an accurate judgment as to the relationship of a suspected organism to a given disease, as, for instance, the demonstration of specific antibodies in abnormally large amounts in the blood of an infected man or animal, or an abnormally high degree of specific immunity to the infecting agent in a recently recovered animal.

It is obvious that technical difficulties may prevent us from fulfilling all Koch's postulates in every case. We may, for instance, be unable to cultivate the causative parasite although we can demonstrate its constant presence in the lesions by suitable staining methods; this has hitherto been the case with regard to the bacillus of leprosy. Or the organism may be so small, and its morphology so doubtful, that we are unable to demonstrate its presence in the tissues, or to differentiate it with certainty from other bodies of the same order of size. If, to this difficulty, is added an inability to cultivate the organism *in vitro*, we may, as in the initial studies on most virus infections, be compelled to take a short cut, neglect the first two postulates, and rely on the reproduction of the disease in a suitable animal by the inoculation of filtrates from infected blood or tissue extracts,

which are at least free from intact cells or living bacteria. In other cases the third postulate may be our stumbling-block. We may be able to demonstrate a characteristic bacterium in such a relation to the lesions of a particular disease that we can have no reasonable doubt that it is playing a causative rôle, and we may have learned how to propagate it in pure culture outside the body; but we may be quite unable to produce in any experimental animal lesions which bear any close resemblance to those which characterize the disease in man.

It is often possible, in the absence of complete proof, to establish the causative rôle of a particular organism with so high a degree of probability that there need be no hesitation in accepting this association as a reasonable provisional hypothesis. At the same time it must be recognized that any omission in the complete chain of evidence involves a risk of error; and much confusion has been caused by uncritical attempts to support the claims advanced on behalf of numberless bacteria isolated from different parts of the body in various diseases.

A little more may usefully be said with regard to the first and third postulates. In the early days of bacteriology the proposition that the causative organism of a disease should be present in every case adequately examined was held to imply its converse—that the organism should not be present in persons not suffering from the disease. And in some instances, such as the investigations that followed Loeffler's demonstration of the diphtheria bacillus, serious doubts were thrown on the claim of an authentic causal organism when it became clear that it could be isolated from the throats of persons who were quite certainly not suffering from diphtheria. Nowadays our conception of the probable distribution of any pathogenic parasite is that it will be found in every case of the specific infection that is clinically obvious and is adequately investigated under satisfactory conditions; in many cases of minor infection that show little resemblance to the fully developed disease; and in a certain proportion of apparently healthy persons, especially in contacts.

With regard to the third postulate, we should not expect complete identity between the clinical picture in man and the lesions produced in an experimental animal. The picture presented by any infective process depends as much upon the reactions of the host's tissues as upon the activities of the infecting parasite; and in this, as in other respects, one animal species differs from another. Sometimes this is a small matter. No one who had any knowledge of the morbid anatomy of tuberculosis in man or cattle would hesitate in identifying the experimental disease in the rabbit or guinea-pig, in spite of minor superficial differences. But the lesions produced in experimental animals by the typhoid bacillus, or by the scarlatinal streptococcus, bear little obvious resemblance to the corresponding human diseases.

The student should note very carefully that there are two distinct stages in the use of experimental animals in the study of infective disease. In the first we are trying to reproduce a particular infection in a form which is clinically or pathologically recognizable as identical with the natural disease. This is the kind of reproduction demanded by Koch's third postulate. In the second we are utilizing our susceptible animal for an intensive study of the particular infective process with which we are concerned. This is really the more significant and interesting part of our investigations. The lesions produced in the guinea-pig by the subcutaneous inoculation of cultures or filtrates of the diphtheria bacillus bear little resemblance to diphtheria as it appears in man; but almost the whole of our present knowledge of immunity in relation to that disease, which has at last placed us in a position to exert effective control, has been built up on knowledge gained from experiments on that small laboratory animal.

It should be noted, also, that there is a shifting of emphasis from the clinical to the bacteriological side as our knowledge progresses. At the start of our inquiry

we are presented with a clinical or epidemiological phenomenon, and our task is to discover the microbial parasite that is the necessary cause. We may, as in the case of diphtheria, or syphilis, or tetanus, find that a single specific parasite is responsible; indeed this is far the most frequent solution if we neglect racial variations within the causative microbial species. We may, however, find that a disease characterized by one clinical syndrome may be caused by one of several distinct species of bacteria. Secondary pneumonia affords one example, bacillary dysentery another. Once the connection between a particular parasite and a particular disease has been firmly established, the tendency of the bacteriologist is to focus his attention on the organism, rather than on the clinical condition to which it most frequently gives rise, and to try to build up a picture of the reactions between that organism and a susceptible host species in general. Thus we should think in terms of pneumococcal infection, rather than in terms of pneumonia; and regard that particular condition merely as one example of the reaction between the pneumococcus and the human tissues, including in our generalized picture such lesions as middle-ear disease, sinusitis, meningitis, peritonitis, suppurative arthritis, etc., and keeping constantly in mind the part played by bacteræmic spread and selective localization.

It will be convenient, in this and the immediately succeeding chapters, to deal exclusively with bacterial infections, since most of our knowledge has been acquired in studying infections of this type. In a later chapter we shall discuss how far our conceptions have to be modified in the case of infections with the filtrable viruses. Protozoal and helminthic infections are beyond the scope of this book; though the methods elaborated by the bacteriologist are proving fruitful in their application to this wider field.

Invasion of the Tissues by Bacteria.

With very few exceptions, bacteria and other microbial parasites must gain access to the host's tissues and multiply in them before they can produce any harmful effect; and we may for the moment regard the capacity to pass through some protective covering layer—most commonly through one of the mucous surfaces of the body—and to multiply in the subjacent tissues, as an essential attribute of a pathogenic organism. The " passing through " must not, of course, be interpreted too literally, or as necessarily implying any active motion on the part of the parasite. Apart from gross mechanical injuries to the skin and mucous membranes, the routes by which micro-organisms and inert particles of non-living matter get into the tissues are probably the same. The skin and mucous membranes of the mammalian body are liberally supplied with lymphatic channels, and access through these channels appears to be particularly easy in those areas where they are near the surface, as in the nasopharyngeal region, the tonsils, and the lymphoid follicles of the intestine. In other cases bacteria may reach the crypts of mucous glands, and then, after local multiplication, penetrate to the subepithelial tissue. In any event it is probable that the capacity for invasion depends in considerable part on a capacity to withstand the conditions existing on the particular surface where lodgment is first made, or in the channels or viscera through which the parasite must pass before it gains contact with a penetrable surface. Little is known of the first stage of infection, the transit of the infecting agent from the outside world to the host's tissues, and the significance of much of it is not always obvious. It will be more convenient to deal with this aspect of the question

in a separate chapter, and to pass at once to the events which follow the passage of the parasite into the tissues.

A broad distinction can be made between those parasites which tend to remain localized at or about their point of entry, and those which are rapidly disseminated throughout the body and produce a generalized infection associated with a bacteræmia. It is unlikely, as we shall see later, that any bacterial parasite multiplies freely in the blood stream, and the presence of large numbers of bacteria in the circulating blood would seem, in most cases, to be the expression of a loss of equilibrium; either a rapid overflow of organisms from extravascular foci of infection overwhelms the clearing mechanisms of the blood, or the clearing mechanisms are themselves put out of action.

The property of invasiveness is one of the characters which distinguish one bacterial species from another, or one strain from another within the same species. Since, in many cases, the pathogenicity of a particular bacterial parasite appears to be mainly determined by its relative rapidity of spread within the tissues, it is logical enough to refer to those species which spread rapidly as *virulent* and to those which spread slowly, or not at all, as *avirulent*; and this usage is generally followed. The term *virulent* is sometimes used as though it were completely synonymous with *invasive*; but this usage is unjustified by derivation and singularly inconvenient in practice. If rigidly adhered to it would necessitate the exclusion from the class of virulent bacteria of all those organisms that exert their lethal effect by the production, in localized foci, of powerful toxins. We could, for instance, no longer speak of virulent and avirulent diphtheria bacilli. It is better practice to retain the term *virulent* in its correct sense of *poisonous*, without any implication as to how the poisonous effect is produced, and to apply it to any organism which gives rise to a rapidly fatal infection. The particular attributes on which virulence depends can be described in other terms.

It may, then, be stated that multiplication of bacterial cells within the tissues is one of the essential factors on which infection depends. Some species can produce a fatal infection without any wide dissemination within the body, others cause death only when such widespread dissemination occurs. A part of the mechanism of resistance will clearly be concerned with the killing and removal of the bacterial cells as such.

Bacterial Localization.

Apart from the generalized bacteræmic invasion referred to above, most bacteria tend to lodge and multiply in tissues remote from their point of entry to the body; and certain species, or groups of species, differ sharply from one another in regard to the particular tissues or organs in which such localization occurs.

The site of the primary lesion and the direction of the initial spread of infection are largely determined by the portal of entry to the body, and by the local anatomy, particularly by the direction of the lymph flow. But the portal of entry in its turn is determined by the natural habitat of the parasite, and this by its potentialities for life under different environmental conditions.

Thus, some types of staphylococci are normal inhabitants of the skin. Those types, or races, that are potentially pathogenic, may gain access to the underlying tissues by the hair follicles, or by the ducts of the sebaceous glands, and cause the characteristic pustules or boils.

Many of those bacteria that lead a parasitic existence in the nasopharynx, and are spread from host to host by droplet infection, tend in consequence of this common portal of entry to show a rough similarity in the distribution of the lesions they

produce; but the similarity is very incomplete, and breaks down altogether on a close analysis. Thus, pneumococci, hæmolytic streptococci, influenza bacilli, diphtheria bacilli and meningococci are all spread by droplet infection. Diphtheria bacilli and hæmolytic streptococci both tend to lodge and multiply in the tonsils, the former giving rise to the characteristic lesions of faucial diphtheria, the latter to tonsillitis. Both produce soluble toxins, which diffuse widely from the initial lesion. But the tendency to invasive spread is much greater in the case of the streptococcus than in that of the diphtheria bacillus. Pneumococci, hæmolytic streptococci and influenza bacilli may all spread to the lungs and cause pneumonic lesions, but their rôle in this regard is very different. The pneumococcus fully merits its name by its pre-eminence as an invader of the pulmonary tissue. It is the sole important bacterial agent in the primary pneumonia of adolescence and adult life. In the secondary pneumonias associated with such infections as measles, whooping cough and influenza, it shares its honours with the influenza bacillus and the hæmolytic streptococcus. The meningococcus, although it is a nasopharyngeal parasite and spreads by droplet infection, produces its characteristic lesion in the meninges. Pneumococci, hæmolytic streptococci and influenza bacilli may also give rise to meningitis; but whereas this is the modal reaction of the meningococcus, when it produces clinically obvious infection, it is a relative rarity among the host of pathogenic reactions caused by the three other bacterial parasites.

The common sites of the primary lesions of syphilis are obviously determined by the venereal mode of infection; but the secondary and later stages are the same whether the primary chancre was genital or extragenital, and the tissues affected and the lesions produced are highly characteristic of the disease.

These instances serve to emphasize that the distribution of infective lesions depends in large part on the biological characters of the infecting parasite, and their relation to the various local metabolic processes in the different tissues of the part.

The intravenous injection of the bovine tubercle bacillus into rabbits, for example, produces visible lesions in the kidney, but none or few in the liver or spleen. Yet in the early stages of the infection, bacilli proliferate equally in all these organs (Lurie, 1928, 1929, 1933). In the guinea-pig, on the other hand, tubercles flourish in lung, liver and spleen. These variations in the susceptibility of organs have their counterpart in variations in the susceptibility of different classes of cells to infection. The studies of Goodpasture and his colleagues on the spread of experimental infection in the embryo of the chick have revealed striking differences in the mode of proliferation of different bacteria (see Goodpasture and Anderson 1937, Gallavan and Goodpasture 1937, Buddingh and Polk 1939, Buddingh and Womack 1941). The proliferation in the embryo of the diphtheria bacillus, the plague bacillus, *Staph. aureus.*, *Str. pyogenes*, the pneumococcus and *H. pertussis* is mainly extracellular; *Str. viridans* and the meningococcus multiply to some extent in the cytoplasm of certain living cells. *Br. tularensis* proliferates intracellularly, mainly in ectodermal cells, and the striking feature of invasion by *Br. suis* is its rapid proliferation in the mesodermal endothelium of the vessels.

The explanation of these variations in organ susceptibility is obscure, though in some cases it may apparently be simply referable to anatomical features. For example, the readiness with which joints are infected after intravenous injection of certain bacteria may be due, not to an inherent arthrotropism of the bacteria used, but simply to the small amount of tissue that separates the cavity of the joint from the rich capillary plexus below the synovial membrane (Shaffer and Bennett 1939). Buddingh (1944) on the other hand, working with a strain of *Streptobacillus moniliformis* isolated from a patient with rat-bite fever (see Chapter 57), found that the organism invaded the chick embryo,

rapidly producing a bacteræmia which was followed by an apparently selective localization in the cells of the synovial linings of the joints, and subsequent invasion of the joint cavity.

The Stimulation of Specific Cellular Reactions.

The differences between the infections produced by the various pathogenic micro-organisms are not confined to the distribution of lesions within the body. The lesions themselves are different and may be highly characteristic. The tendency of certain bacterial species to stimulate a localized polymorphonuclear reaction, associated with pus formation, has earned for them the title "pyogenic." Such experimental evidence as is available indicates that the migration of polymorphonuclear leucocytes to the focus of infection is the direct result of an attractive (chemotactic) effect, exerted by some diffusible bacterial product. Equally characteristic lesions, which differ among themselves, are produced by the various non-pyogenic organisms. The diphtheria bacillus, the typhoid bacillus and the *Treponema pallidum* give rise to tissue reactions which are so distinct that any competent histologist could differentiate them without bacteriological assistance. The special case of tuberculosis is of particular interest, in that a beginning has been made in tracing the connection between the various chemical products of the bacterial cell and the type of tissue reactions to which they give rise (see p. 410).

It is not, of course, the case that every pathogenic bacterium provokes a distinct and characteristic cellular response, or that the pathologist unaided can often arrive at an exact bacteriological diagnosis from a histological examination of the infected tissue ; but, as Kettle (1927) has pointed out so clearly, there is a broad correlation between certain types of lesion and certain groups of bacteria, and the groups which the pathologist would recognize on the basis of the distribution and type of the lesions produced are, in many cases at least, those which the bacteriologist would recognize as containing closely related bacterial species.

All this may seem very obvious and elementary ; but it is of the first importance to realize that different pathogenic parasites have different points of attack, and different methods of attacking.

Bacterial Toxins.

There is no apparent reason why the mere presence in the tissues, or blood stream, of any reasonable number of cells of an order of size expressed in low multiples of μ should exert any harmful effect on the host that harbours them. No mechanical theory of the pathogenic action of bacteria is compatible with our knowledge of the way in which the tissues deal with inert particles which have gained access to them. The basis of all harmful effects of bacterial infection is quite certainly chemical ; and only when the chemist has replaced the immunologist shall we be able to give an intellectually satisfying account of what happens when a particular parasite invades a particular host. In the meantime we must do what we can with the crude data at our disposal.

It has long been known that certain bacterial species produce highly poisonous substances which give rise to characteristic lesions or symptoms when injected into susceptible animals. These are the bacterial toxins. A convention has become established according to which those toxins which diffuse readily from the bacteria that produce them are referred to as *exotoxins*, while poisonous substances which remain attached to the protoplasm of the bacterial cells are referred to as *endotoxins*. As knowledge has accumulated, it has been possible to correlate this character with

others, and a broad distinction may be made between characteristic members of the two groups.

A typical exotoxin is readily separated from a bacterial culture, in a suitable fluid medium, by simple filtration through a candle of porcelain or diatomaceous earth. It shows a characteristic affinity for certain cells of a susceptible host, producing easily recognizable symptoms during life, or lesions which can be detected after death. Thus one exotoxin can usually be distinguished from another by what we may term its pharmacological action. Instances of this specific effect are afforded by the action of tetanus toxin on the motor nerve cells, by the ocular and pharyngeal paralyses caused by the toxin of *Cl. botulinum*, and by the cardiovascular failure, the hæmorrhagic lesions of the adrenals, and the late paralysis in animals that survive, which are a characteristic of experimental diphtheritic toxæmia.

An exotoxin is usually fatal in a very small dose. The *Minimal Lethal Dose* of a potent diphtheria toxin for a guinea-pig is about 0·002 ml., of a potent tetanus toxin about 0·0005 ml., and of a potent botulinum toxin about 0·0001 ml. Such toxins are usually relatively thermolabile. Diphtheria toxin is inactivated by heating at 58°–60° C. for 1–2 hours; tetanus toxin by heating at 60° C. for 20 minutes. Lastly—and this is a matter which will be considered more fully in a subsequent chapter—exotoxins are usually highly antitoxinogenic; that is, on inoculation into a suitable animal they stimulate the production of antitoxins which neutralize them specifically; and broadly speaking the amount of antitoxin needed for neutralization is directly proportional to the amount of toxin present.

The typical endotoxin is not liberated into the fluid medium in which the bacterium producing it is growing, and hence cannot be separated from the bacterial cells by simple filtration. It is liberated in solution or suspension by various treatments of the cell, alternate freezing and thawing, prolonged grinding, subjection to sonic vibration, extraction with different solvents—or by merely allowing cells to autolyse in a fluid medium. Toxins of this type may in most cases be demonstrated by the injection into a susceptible animal of the unaltered bacterial cells. In this case it is assumed that the liberation of the toxin is brought about by the lysis of the cells *in vivo*. The endotoxins do not, in general, give rise to diagnostic symptoms or lesions in experimental animals, so that it is not usually possible to recognize any particular endotoxin by its pharmacological action. The *Minimal Lethal Dose* of a typical crude endotoxin is in general larger than that of a typical exotoxin; sometimes the ratio is of the order of 1,000,000 to 1. Thus the M.L.D. of a killed broth culture of the cholera vibrio is about 0·5 ml. for the guinea-pig, the M.L.D. of meningococcus for the mouse is about 2 mgm. of bacillary substance, and the M.L.D. of the gonococcus for the same animal is about 10 mgm. of bacillary substance. Endotoxins are usually relatively thermostable. Cholera endotoxin requires 1 hour's heating at 80°–100° C. for its inactivation; the endotoxin of the meningococcus requires 1 hour's heating at 120° C.; while the same treatment has failed to inactivate the endotoxin of the gonococcus. The virulent S form of *Sh. shigæ* produces two main toxic components which will illustrate the differences between "exotoxins" and "endotoxins." The "exotoxin" has a mouse M.L.D. of $< 0{\cdot}001$ mgm., is an actively antigenic thermolabile protein, and induces characteristic paralyses in mice and rabbits. The "endotoxin" has a mouse M.L.D. of about 0·1 mgm., is a thermostable moderately antigenic complex of phospholipin, polysaccharide and conjugated protein, and, though its capacity to produce marked œdema and hæmorrhages in the gut of experimental animals has earned for it the name of "enterotoxin," it does not differ qualitatively in its general toxic action from similar components in other intestinal bacilli.

The endotoxin of a number of other Gram-negative intestinal bacilli, tested in more or less pure preparations, have M.L.D. that vary round 0·1 to 1·0 mgm.

Finally, endotoxins are seldom strongly antitoxinogenic. In some cases it has proved impossible to prepare anti-endotoxic sera. More commonly prolonged immunization

of an animal yields an antiserum which is able to neutralize a few M.L.D. of the endotoxin; but the simple linear relationship between the amount of toxin and antitoxin in a just neutralized mixture, that obtains with exotoxins, does not hold with endotoxins. In many cases no increase in antitoxin affords protection when the dose of endotoxin passes a certain limit.

Although this broad distinction can be made by comparing typical exotoxins with typical endotoxins, not all toxins fall simply into two well-defined groups. Thus, the toxin of *Cl. botulinum* is a typical exotoxin, filtrable, with a very small M.L.D., with a characteristic pharmacological action, and antitoxinogenic; but it is relatively heat-resistant (75°–80° C. for 10 mins.). The α-toxin of *Cl. welchii* is filtrable, has a characteristic local action on the tissues, and is antitoxinogenic; but its M.L.D. is relatively large (0·02–0·1 ml. for the pigeon), and it is relatively heat-resistant (70° C. for 30 mins.). The erythema-producing toxin of the hæmolytic streptococcus—the toxin which is responsible for the rash in scarlet fever, and is employed in the Dick reaction—is filtrable, has a characteristic action on the skin of a susceptible person, and is antitoxinogenic; but its M.L.D. is very large (5–10 ml. or more of an unconcentrated filtrate for the rabbit) and it is very resistant to heat (96° C. for 45 mins.). The hæmolytic streptococcus also produces another filtrable toxic constituent which differs from the Dick toxin in being thermolabile (55° C. for 30 mins.), in causing lysis of red blood cells, and perhaps of leucocytes, though the identity of streptococcal hæmolysin and leucocidin is not accepted by all observers. Its M.L.D. is large, of about the same order as the thermostable toxin, but in this dose it kills susceptible rabbits within 24–36 hours with an associated hæmoglobinuria and characteristic evidence of intravascular hæmolysis at necropsy (M'Leod and M'Nee 1913, Channon and M'Leod 1929).

One implication which has sometimes—though without justification—been attached to the term "exotoxin" is very probably misleading. Toxins of this type have been described in words which suggest that they are of the nature of active secretions of the bacterial cells. There is little definite evidence that this is so. Though some exotoxins, as that of *Cl. welchii*, are produced in high concentration during the phase of active bacterial growth, others, such as diphtheria, tetanus and Shiga toxins, only attain a high concentration in a fluid culture when the phase of active growth has passed, and the majority of the bacteria present are dead or dying. How far autolytic processes may be concerned in toxin production is quite uncertain. Morton and Gonzalez (1942) compared the amount of toxin in the fluid part of a culture of diphtheria bacilli with that in the cells after disruption by sonic vibrations. Their results indicate a very much higher concentration of toxin in the cells, though the absolute amount of toxin in the fluid was the greater.

Most of the studies on bacterial toxins have been made with crude reagents—filtrates, autolysates or extracts of fluid cultures,—which quite certainly contain many substances other than the toxins. Moreover, it is now clear that a single species of bacterium may elaborate a number of different exotoxins and endotoxins. For instance, filtrates of *Cl. welchii* Type B may contain at least five distinct exotoxins; and two major endotoxins have been isolated from virulent typhoid bacilli.

Constitution of Toxins.—Few of the exotoxins have been characterized chemically. The toxin of the diphtheria bacillus is a heat-coagulable protein, very sensitive to denaturation (Eaton 1937, Pappenheimer 1937, Boivin 1937). Pappenheimer (1938) obtained some evidence that the integrity of the ε-amino groups of the lysine residues in diphtheria toxin was associated with its toxic properties.

Scarlet fever toxin appears to be a protein of low molecular weight (Barron, Dick and Lyman 1941), and both the exotoxin of *Sh. shigæ* (Boivin and Mesrobeanu 1937*a*, *b*) and the O hæmolysin of *Str. pyogenes* have the characters of a protein (Herbert and Todd 1941). A number of endotoxins, on the other hand, have been characterized with some precision. In both the salmonellæ and the dysentery bacilli, the antigens characterizing the smooth virulent phases are also the endotoxins. Boivin and his colleagues (1933–5) and Raistrick and Topley (1931) (see also Delafield 1934, Martin 1934, Boivin 1938) isolated from salmonella bacilli antigenic endotoxins which appeared to be complexes of a polysaccharide and a phospholipin. These glycolipoid complexes were split on mild hydrolysis into a non-toxic polysaccharide, and a feebly toxic lipin. Morgan (1937) isolated a similar substance from *Sh. shigæ*. Morgan and Partridge (1940, 1941) subsequently demonstrated a third component in the complete endotoxin of Shiga's bacillus, a polypeptide which appeared to be a conjugated protein. A similar polypeptide component is also present in the endotoxin of *Salm. typhi* (Freeman and Anderson 1941) which proved to be identical in all respects tested with the conjugated protein of Shiga's bacillus (Morgan and Partridge 1942). The antigenic specificity of these endotoxins is in each case conferred by the polysaccharide component, which, as we saw in Chapter 7, may be regarded as a hapten. Toxic antigens of this type, characterized by different polysaccharide haptens, are widely distributed among the Gram-negative bacilli of the coli-typhoid group, and among other more distantly related bacteria. (For a discussion of mycobacterial and neisserial endotoxins, see Chapters 16 and 23.)

Mode of Action of the Toxins.

It has long been recognized that the healthy tissues of an animal can be poisoned by products of tissue breakdown in some other part of the body. One of the difficulties in determining the mode of action of bacterial toxins is the separation of the secondary effects due to tissue breakdown, from the primary effects of direct intoxication by some constituent of the bacterium. As an example of a secondary effect we may cite " leucotaxine " (Menkin 1938), a substance increasing the permeability of the walls of the blood capillaries, and " necrosin " (Menkin 1943, 1944), a necrosis-inducing substance associated with a protein. Both are products of inflamed tissue, irrespective of the primary inflammatory agent. The comparatively rapid action of some exotoxins, especially when administered intravenously, suggests that we are dealing with a primary effect. If, however, the effect is delayed for a few hours. it is impossible on *a priori* grounds to distinguish a latent period due to a slow penetration of toxin into, or a slow accumulation of toxin in, primarily susceptible tissue, from a latent period during which secondary toxins are being elaborated in primarily intoxicated tissues. The difficulty will probably be resolved by precise physiological and biochemical studies on the action of pure preparations of bacterial toxins. It must be realized, however, that the conspicuous effects that have been obtained in short-period observations of animals injected with relatively large doses of toxin may have little direct bearing on the relatively slow process of intoxication that occurs in natural bacterial infections (Wright 1942).

To postulate that toxins interfere with some essential metabolic processes in the animal tissues is merely another way of saying they are toxic. They may act by poisoning enzymes, rendering substrates insusceptible to enzymes, or altering the quantity or

quality of the products of certain enzyme processes. Both Wooldridge and Higginbottom (1938) and Peters and Cunningham (1941) have tested the effect of exotoxins on enzyme systems isolated from animal tissues. The former showed that the α-toxin of *Cl. welchii* depressed the aerobic oxidation of succinate by guinea-pig intestine, but only when present in very high concentration. Peters and Cunningham observed no change in a number of heart-muscle dehydrogenase systems to which diphtheria toxin had been added. Diphtheria induces in the carbohydrate metabolism of the rabbit changes attributable to abnormally large secretions of adrenaline (Corkill 1932, Corkill and Ochoa 1934, Yannet and Darrow 1933). Cross and Holmes (1937) demonstrated that isolated liver tissue from rabbits so intoxicated exhibited diminished respiration and diminished capacity to store glycogen and to synthesize carbohydrates from compounds like pyruvic and lactic acid. They were unable to decide whether the alterations were due to a direct damage of the liver cells, or to modification of the hormonal influences to which they are normally subjected.

Some of the specific physiological effects of the exotoxins of *Cl. welchii* in the whole animal have been studied by Kellaway and his colleagues (Kellaway, Trethewie and Turner 1940, Kellaway and Trethewie 1941). It is sufficient to note here that they range from direct action on the central nervous system, and the peripheral arterioles, to the liberation from tissues of histamine, adenyl compounds, and a substance causing slow contraction of the involuntary muscle. The α-toxin of *Cl. welchii* is of particular interest, being the first toxin whose action could be attributed directly to its enzymic nature. It is a thermostable lecithinase (Macfarlane, Oakley and Anderson 1941, Macfarlane and Knight 1941) to whose power of splitting lecithin and lecithoprotein complexes the α-toxin probably owes its hæmolytic, necrotizing and lethal properties.

The glycolipoid or, as they may be called, the lipo-polysaccharide endotoxins of the Gram-negative bacilli described above appear to act on the animal body in the same way, irrespective of the particular bacterium from which they are derived.

For example, injection of whole bacteria or of bacterial products is followed by changes in the concentration of blood sugar. The earlier findings in this respect (Menten and Manning 1925, Zeckwer and Goodell 1925, Menten 1926, Levine and Kolars 1926, Menten and King 1930) are difficult to correlate and interpret. Delafield (1931, 1932) found that intravenous injection of killed suspensions of various Gram-negative bacteria was followed by a marked hyperglycæmia, maximum in a few hours, then declining into a hypoglycæmic phase marked at 24 hours, after which, if the animal survived, the blood sugar returned to normal. The inorganic phosphorus levels fluctuated inversely as the blood sugar levels. Gram-positive bacteria do not appear to contain substances of this type (Delafield 1932, Olitzki, Avinery and Koch 1942). The endotoxins, as subsequent work has shown, are responsible for this effect (Delafield 1934, Boivin and Mesrobeanu 1934*b*, Morgan 1941, 1943, Favorite and Morgan 1942, Olitzki, Bendersky and Koch 1943, Leon and Morales 1943). The other non-specific effects of these endotoxins, depending on the dose and animal tested, include leucopenia, followed by a polymorphonuclear leucocytosis, and endothelial damage resulting in hæmorrhages, and sometimes focal necroses.

It is possible that adrenal stimulation and damage may play some part in the production of these effects (see Evans and Zeckwer 1927, Olitzki, Avinery and Koch 1942). In *Sh. shigæ* Olitzki and his colleagues (Olitzki, Leibowitz and Berman 1937, Olitzki and Avinery 1937) distinguish a toxic fraction and separate fractions producing leucopænia, glycæmia and hypothermia respectively. It is not certain how far their fractions were made up of complete endotoxin, and of breakdown products therefrom.

Other Active Constituents or Products of Bacteria.

We have already noted certain specific or non-specific biological characteristics of toxins; and many others, like the hæmolysins and leucocidins of *Staph. aureus*, could be instanced. In this section we are concerned with bacterial products

that are not markedly toxic in themselves, but act upon animal tissues and fluids in such a way as to suggest that they may influence the course of a bacterial infection. It is to be remarked, however, that the mere association of a biochemical property and pathogenicity either in a bacterial species or in the more virulent strains of a pathogenic species, is not by itself evidence that the property is concerned in infection by the species. As we have seen in the chapter on variation, latent activities of bacteria can be made manifest by the provision of suitable substrates, and though a virulent bacterium may exhibit an activity because a suitable substrate is present in the tissues of its host, it does not necessarily follow that the virulence depends on the capacity to attack the particular substrate; the capacity may in this respect be purely accidental.

Thus among the staphylococci, the more pathogenic *Staph. aureus* produces a coagulase that accelerates the clotting of human and rabbit plasma (Much 1908, Gross 1931*a*, *b*, Gengou 1933, Chapman *et al.* 1934). Since the formation of fibrin clots is a marked feature of *Staph. aureus* lesions in man and animals, it is natural to relate the two features. Nevertheless, Menkin and Walston (1935) could find no evidence that staphylocoagulase was concerned in the production of the staphylococcal lesions.

Tillett and Garner (1933) demonstrated an antigenic substance in hæmolytic streptococci that dissolves human fibrin (see also Tillett, Edwards and Garner 1934, Tillett 1935, 1938, Jablonowitz 1939). This fibrinolysin, though relatively heat-stable, behaves in many ways like an enzyme (see Mirsky 1944). It is produced by most Group A, C and G streptococci associated with suppurative and invasive types of human infection, while strains from feebler infections and throat-carriers are less actively fibrinolytic.

There is no doubt that active substances of the coagulase and fibrinolysin type are produced by a considerable variety of bacteria (see, for instance, Reimer 1936, Saski and Fejgin 1937, Christie and Wilson, 1941, Reed *et al.* 1943); and that a single bacterium can produce both types of substance. Again, each type of activity shows some degree of host specificity. Staphylocoagulase is inactive in guinea-pig plasma, and streptococcal fibrinolysins do not dissolve rabbit or fowl clots (see also Le Mar and Gunderson 1940). Milstone (1941) has shown one way in which this species-specificity may be determined. He found that human, but not rabbit, serum contains a heat-labile " lytic factor " associated with the euglobulin, in the absence of which streptococcal fibrinolysin failed to dissolve a pure fibrin clot. If the lytic factor were added to the rabbit fibrin, it became susceptible to the fibrinolysin. Smith and Hale (1944) found that susceptibility of sera to staphylocoagulase also depended on the presence in the serum of an activator. Human and rabbit sera contain the activator, which, when added to naturally insusceptible guinea-pig serum, renders it susceptible to coagulase. For the most part these activities are broadly associated with virulence, but highly virulent strains may not possess them, and vice versa; Sartorius (1936), for instance, could find no correlation between fibrinolytic activity and virulence in hæmolytic streptococci.

One of the most interesting classes of these relatively non-toxic bacterial products is that of the " spreading " or " diffusing " factors. Duran-Reynals (1928, 1929) described a substance in extracts of bull testicle that increased the area of the lesions following the injection of infective material into mammalian skin. As McClean (1930) and Hoffman and Duran-Reynals (1931) showed, the substance increased the permeability of the tissues, facilitating the spread of the fluids injected—whether solution of dye or toxins, a suspension of Indian ink, viruses or bacteria. Intradermal injection of fluid ordinarily raises a bleb that may take several hours to disappear. The presence of diffusion factor causes the bleb to disappear within a few minutes.

We can indicate only the broader relations of diffusing factors to infection. For detailed information the reader is referred to the review by Duran-Reynals (1942). The addition of diffusing factor to an inoculum of pathogenic bacteria or viruses enhances

the lesion only if the concentration of infectant is relatively great, or if its virulence is relatively high. If, for example, the bacteria are present in small numbers, or are only moderately virulent, the lesion may not develop at all, while injection without diffusing factor gives rise to a circumscribed lesion. Clearly the diffusing factor may inhibit the appearance of a lesion by spreading the pathogenic material in subinfective concentration throughout the tissues (see Thomas and Duran-Reynals 1935). On the other hand, if the bacterium is highly virulent, the lesion is not only larger than that produced in the absence of diffusing factor, but may have a more uniformly fatal outcome. Diffusing factors are elaborated by certain species of bacteria; *Staph. aureus* and *Str. pyogenes* (Duran-Reynals 1933); pneumococci (Goodner 1931, 1933); various clostridia and some strains of diphtheria bacilli (McClean 1936). Diffusing factor has not been found in members tested of the *Bacterium, Pasteurella, Rickettsia, Bacillus, Brucella, Vibrio, Hæmophilus* and *Neisseria* groups (see also Ungar and Bacharach 1942).

The identity of certain mammalian and bacterial diffusing factors with the enzyme hyaluronidase was first suggested by Chain and Duthie (1940), and although the parallelism is not complete (see Hobby *et al.* 1941, Meyer *et al.* 1941) the fact that many bacterial diffusing factors exhibit hyaluronidase activity and that growth of certain bacteria in media containing hyaluronic acid enhances the production of diffusing factor and hyaluronidase to the same degree (McClean 1941, McClean and Hale 1941) strongly suggests that the diffusion factors act by virtue of contained hyaluronidase. The intercellular ground substance of mesodermal tissue contains hyaluronic acid in a viscous polymerized form, and it appears that the enzyme hydrolyses the viscous polymer and thus permits the ready diffusion of fluid through the intercellular spaces.

It is evident that in certain experimental infections, the addition of hyaluronidase to the inoculum enhances the virulence of the contained organisms. The rôle of hyaluronidase in infections by organisms that produce it themselves is less clear. McClean and his colleagues (1943) detected large amounts of hyaluronidase in well-established local lesions by *Cl. welchii, Cl. septicum* and certain strains of *Cl. œdematiens*, and it is reasonable to postulate that in such circumstances the spread of the hyaluronidase-producing organisms would be facilitated by the enzyme. But it may be argued that, in the very early stages of a natural infection when the growing bacterium is present in small numbers, hyaluronidase, by promoting a rapid dispersal of the bacteria through healthy tissues, would expose invading organisms more thoroughly to the lethal action of the tissues.

The production of hyaluronidase, like that of coagulase and fibrinolysin, is associated with virulent species of bacteria, and to some extent with the virulent strains within a species. There is not, however, a complete correlation between invasiveness and hyaluronidase production. For example, some highly invasive strains of *Cl. welchii* produce none (Evans 1943); and the same is true of certain types of *Str. pyogenes* obtained from severe human infections (Crowley 1944). Moreover, bacteria like *Brucella melitensis* and *tularensis* and *Pasteurella pestis*, which are highly invasive in the accepted sense of the word, produce no hyaluronidase, while *Staph. aureus*, which is not markedly invasive, produces it in abundance. The rôle of hyaluronidase in infection cannot be settled until it is quite clearly shown that the virulence of a strain producing the enzyme is significantly altered by inhibition of its hyaluronidase-producing powers, or by neutralization of the hyaluronidase *in vivo*. Evans (1943) has made a start in this direction by showing that passive immunization of guinea-pigs with an anti-hyaluronidase serum does not materially alter the capacity of minimal infecting doses of *Cl. welchii* to engender gas gangrene, though his results are open to the criticism that the antiserum employed was too weak to exert a protective effect.

It is probable that pathogenic bacteria produce enzymic and other non-toxic substances which, by their action on the tissues influence the course of an infection (see, for example, Birch-Hirschfeld 1940). But in all cases we must guard against confusing an association with a causal linkage. As we have already emphasized,

a biochemical character may be genetically linked with characters that determine the virulence of an organism, and at the same time play no significant part in infection.

It seems quite possible that, wherever there is formed in the tissues a nidus of bacterial cells, alive or dead, there may result in the immediate neighbourhood a diversion of the normal metabolic processes sufficiently serious to derange the local cell activities. It is not impossible that the diffusion of bacterial enzymes might convert such a local effect into a more general one.

We should then regard the bacterial cell as endowed with an extensive armoury of chemical weapons of attack. These include (*a*) the filtrable toxins, diffusing freely from the site of infection and acting specifically on certain sensitive tissue cells. These are often lethal in extremely small doses; (*b*) the hæmolysins and leucocidins, acting directly on red cells and leucocytes, and hence influencing the course of an infection; (*c*) toxic substances contained in the bacterial cells, and not freely liberated into the surrounding fluid. Among these endotoxins are some, at least, of the somatic bacterial antigens, that are lethal in moderate doses and induce characteristic changes in the chemical constitution of the circulating blood; (*d*) various active substances which, though not directly toxic, may possibly influence the reactions of the tissues by inducing or inhibiting the clotting of plasma or lymph or by dissolving the fibrin of a clot already formed, or by increasing the permeability of the tissue spaces, or by any combination of these effects.

Hypersensitiveness and Allergy.

There are reasons, which will be discussed more fully elsewhere, for believing that the tissues may become sensitized during the course of a bacterial infection, so that a bacterial constituent which before called forth little or no response may act as a powerful stimulus. In some cases the vigorous response evoked is injurious to the host, so that a bacterial product which possesses little or no toxicity for a normal animal may be highly toxic for an animal which has become sensitized by infection. The well-known tuberculin reaction in the guinea-pig is a case in point.

Some Illustrative Examples.

We have described above the various bacterial factors which, so far as we know at the moment, are certainly, probably, or possibly concerned in the pathogenesis of infective diseases.

A few illustrative examples may serve to emphasize the more important points.

Botulism may be placed at one end of the scale, as an example of a pure toxæmia. It is not a true infection, but is closely analogous to the effects that may follow the consumption of such vegetable poisons as those produced by certain fungi or plants. There is no certain evidence that *Cl. botulinum* ever invades the tissues, or multiplies in them. Man is affected by eating food in which the organism has grown and produced its toxin.

Tetanus is one step removed, in that the bacillus must gain access to the tissues and multiply in them in order to produce its toxic effects; but its invasive powers are very slight. It usually gains access to the body as the result of some mechanical injury, and the local multiplication of the organism is in many cases assisted by gross injury to the tissues, by the injurious effects of some associated infection, or by the presence of some chemical agent, such as a calcium salt, which alters the local conditions to the advantage of the parasite.

Diphtheria takes us a little further along the scale. The bacillus is capable of implanting itself in the faucial region, and of spreading from host to host, by ordinary droplet infection or in similar ways. It has relatively slight but quite definite invasive powers. It causes a characteristic local lesion, and may occasionally invade other tissues and organs. Its lethal effect, however, depends almost entirely on its activity as a toxin-producer.

The hæmolytic streptococcus combines the activities of a typically toxigenic organism with those of the highly invasive group of bacteria. It produces a relatively heat-stable toxin, with a characteristic action on the skin, which is the cause of the typical toxic rash in scarlet fever; but it also invades the tissues freely. Indeed, highly virulent strains of the organism are among the most rapid and vigorous tissue-invaders with which we are acquainted in human pathology. In relation to this tissue invasion it is very probable that the hæmolysin, the leucocidin, and perhaps the fibrinolysin and the hyaluronidase produced by pathogenic streptococci play a significant part.

As we move along the scale we meet a variety of organisms whose pathogenicity seems to depend entirely on their power of spread and multiplication in the tissues, and whose more important effects seem to be localized to the areas in which such multiplication occurs. These differ among themselves in selective localization, and in the tissue reactions to which they give rise. Thus, staphylococci most commonly cause localized suppurative lesions, with a varying degree of lymphatic spread, which may sometimes be rapid and extensive. More rarely they give rise to a generalized infection of the pyæmic type, in which the cocci are carried from the original focus of infection in detached fragments of an infected thrombus. Here again, hæmolysin, leucocidin, and perhaps coagulase, fibrinolysin and hyaluronidase may play a part in determining the type of lesion in any particular case.

Finally, there are organisms, such as the tubercle bacillus, the leprosy bacillus and the *Tr. pallidum*, that tend to produce more chronic lesions of the granulomatous type. We know little as yet of the bacterial constituents that determine this particular type of tissue response, except in the case of the tubercle bacillus—see Chapter 59; and even there our knowledge is as yet very scanty. It seems probable that it is conditioned in large part by the absence of those bacterial constituents that determine the more rapid and vigorous response induced by organisms of the acutely toxic, pyogenic, or invasive type.

The defence mechanisms brought into play, and their relative importance, will clearly differ according to the type of infection. It is with these mechanisms that we shall be concerned in the following chapters.

SUMMARY

(1) An ability to gain access to the tissues, and to multiply in them to a lesser or greater degree is an essential attribute of a pathogenic organism. Some organisms produce their effects by the local production of a powerful toxin which passes into the tissues, and produces its toxic effects on cells remote from its site of origin. Others produce their main pathological effects at the site, or sites, in which they are multiplying. Of these latter, some give rise mainly to lesions limited to the near neighbourhood of their point of entry; others to lesions remote from this point, but characteristically localized in certain tissues or organs; others, again, to a

generalized bacteræmic infection. These different modes of attack are often combined in the case of a single infecting bacterium.

(2) The selective localization of bacteria in particular organs or tissues of the body is determined in part by characters inherent in the bacteria themselves. The anatomy of the infected host, the biochemical reactions of different tissues, and the defence mechanisms brought into play, combine with these inherent bacterial characters in determining the exact distribution of lesions encountered in each particular case.

(3) Pathogenic bacteria differ in the cellular reactions to which they give rise. These differences may be highly characteristic.

(4) Among the bacterial toxins are some which are characterized by the ease with which they may be separated from the bacterial cells which produce them. Under suitable conditions they diffuse into a fluid medium and may be obtained from the cells by simple filtration. Toxins of this class are commonly referred to as *exotoxins*. They are usually characterized by possessing a specific pharmacological action, and by being actively antitoxinogenic. Their Minimal Lethal Dose is generally small, sometimes very small. They are in many cases thermolabile, being inactivated by temperatures in the neighbourhood of 55° C. The more fully investigated exotoxins are predominantly protein in nature.

(5) Apart from these toxins there is another class of toxic bacterial products which are characterized by remaining incorporated in the substance of the bacterial cells. They can be obtained in a state of solution, or perhaps suspension, only by extraction or manipulations that result in the disintegration of the cells. They do not, in general, exert any characteristic pharmacological action by which they can be differentiated from one another. They are feebly antitoxinogenic. Their Minimal Lethal Dose is usually large. They are relatively heat-stable, withstanding temperatures of 80°–120° C. for an hour or more. They are often referred to as *endotoxins*. The more fully investigated endotoxins have proved to be predominantly lipo-polysaccharide in nature; some of them contain protein-like substances that are essential to the constitution of the toxic complex.

(6) Although it is often an easy matter to assign a toxic bacterial product to one of these categories, this is not always the case, since the criteria referred to above are by no means constant. It would, indeed, be very difficult to give any satisfactory definition of an endotoxin. Within this category we may include certain of the somatic antigens of bacteria that have been found to be highly toxic in the form in which they occur in the bacterial cell.

(7) There are other substances in bacterial cells which are not in themselves toxic, but whose nature—the capacity to induce or inhibit the coagulation of plasma or lymph, to dissolve a fibrin clot, or to increase the local intercellular permeability of tissues—suggests that they may influence the course of the infective process; and it is possible that other enzymes concerned in bacterial metabolism may have a like influence in infection by alteration of the metabolism of the tissues.

(8) As the result of infection the tissues of the host may become hypersensitive to the infecting bacteria or to their products. Such sensitization may play an essential part in some of the phenomena of infective disease.

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CHAPTER 45

THE MECHANISMS THAT HINDER OR PREVENT THE ACCESS OF BACTERIA TO THE TISSUES

As noted in the previous chapter, an ability to gain access to the tissues and to multiply in them to a lesser or greater degree is an essential attribute of a pathogenic organism. It is logical to commence our study of the defence mechanisms of the host by considering the barriers that are opposed to the ingress of bacterial parasites from the outside world. Unfortunately our knowledge is here at its sketchiest. Most studies of experimental infections in animals have been concerned with reactions that take place within the tissues. It is only within recent years, and in the hands as yet of relatively few workers, that any serious attempt has been made to study systematically the mechanisms concerned in the first line of defence.

It will be convenient to take in turn the various body surfaces and portals of entry, and to summarize very briefly our scanty knowledge of their behaviour towards bacteria that establish contact with them.

The Skin.—It needs only the most casual acquaintance with the ordinary accidents of life to establish the value of an intact skin as a barrier against bacterial infection. The inability of the deeper tissues to protect themselves against the free access of bacteria from the outside world set a sharp limit to major surgical interference in pre-Listerian days. There can be little doubt that the skin functions in the main as a simple mechanical barrier, but it has recently become clear that the efficacy of this barrier is enhanced by a complex biological mechanism which enables the skin surface to free itself rapidly from the majority of those bacteria that are continually impinging upon it.

The skin has certain self-sterilizing powers. Colebrook (1930) found that hæmolytic streptococci were rapidly killed on the skin of the normal hand. Three minutes after swabbing the finger lightly with a broth culture, 30 million cocci could be recovered by swabbing with sterile broth. One hour later 1·7 million cocci were recoverable; two hours later, 7,000 cocci. Similar results were obtained with *Proteus vulgaris*, *Bact. friedlanderi* and *Bact. coli.*, and control experiments on other surfaces showed that the bacteria had not died by desiccation. Though the skin will free itself to a considerable extent from bacteria that form no part of its normal flora, its reactions are entirely different towards those species that habitually live on it. No amount of washing, nor any of the chemical disinfectants commonly used for sterilizing the hands in obstetrical practice, will remove from the skin the numerous staphylococci and bacilli that normally vegetate thereon. These organisms, which were named by Price (1938) the "residents," as opposed to the "transients" like *Str. pyogenes*, probably lie in the ducts of the sweat and sebaceous glands, in hair follicles, or among the cells of the stratum corneum, though Price himself suggests that they are in some intimate manner adherent to the skin surface.

Arnold, Gustafson and others, 1930 (see also Karns and Arnold 1931), record a more detailed series of observations along similar lines. About 99 per cent. of *Chromobacterium prodigiosum* could not be recovered in 20 minutes, and all were dead within 30 minutes. On the palmar surface of the clean hand *Bact. coli*, *Salm. typhi* and *Salm. enteritidis* could not be detected after 10 minutes, though in certain regions, under the nail-tip and along the lateral margins of the finger nails, the bacteria persisted longer. There was a rapid initial reduction of *Staph. aureus* on the palmar surface of the hand, but small numbers persisted for long periods. The skin of dirty hands had less self-sterilizing power, the reduction of *Salm. enteritidis* being 5 per cent. in 20 minutes; after washing the reduction was 100 per cent. in 20 minutes. Fifteen minutes after the death of the skin, most of the bactericidal action for *Bact. coli* had disappeared

The readiness with which *Str. pyogenes* is killed on the skin has been partly elucidated by Burtenshaw (1938, 1942) (see also Williams *et al.* 1943) who extracted, from normal living or dead skin, substances resembling long-chain fatty acids and soaps that were markedly bactericidal for *Str. pyogenes*, but not for *Staph. aureus*.

Arnold and his colleagues attach considerable importance to the effect on bacteria of the pH of the skin, which is usually acid (5·8–5·2, see Schade and Marchionini 1928). Marchionini and others (1938), using a skin electrode, found the greater part of the skin surface was acid, the pH in some spots being as low as 5 or even 3. There were alkaline areas in the axillæ and groins, between the toes and on parts of the foot, which they regarded as gaps in the "acid coat" that constitutes a defence against infection of the skin. The flora of skin was scantier in the acid areas and, in these, applied *Chromobacterium prodigiosum* disappeared more rapidly than in the alkaline areas. After induced sweating the skin increased in acidity, and was more bactericidal. Bergeim and Cornbleet (1943) attribute the acidity to the lactic acid excreted in sweat, and to the volatile acids formed from it by bacterial action. The former is active in regions where the pH of the skin is 5 3 or less; at a higher pH, it dissociates and loses much of its bactericidal power. The latter are active at a pH of 5·3 to 6·2, and will consequently be effective in the more alkaline areas of the skin.

(For general discussion of the protective powers of the skin see also Vierthaler 1940, Neufeld 1942).

It should be noted that not only may bacteria be killed, but viruses may be inactivated on skin. Influenza virus, for example, in one series of experiments, could not be detected 10 minutes after application to the human skin (U.S. Navy Medical Bulletin 1943); though in another series (Parker and MacNeal 1944) suspensions of virus in the allantoic fluid of the chick survived 45 to 50 minutes on the skin of the hand.

Fleming (1922, 1929, 1932) has recorded the presence in tears, in nasal secretion, and in many tissues including the skin, of a relatively thermolabile substance which, in high dilution, causes the lysis of certain non-pathogenic bacteria, and in particular of an organism to which he gave the name of *Micrococcus lysodeikticus*. This substance, *lysozyme*, is an enzyme (Meyer *et al.* 1936, Abraham 1939) which appears to initiate lysis by splitting certain polysaccharide components of susceptible bacteria (Epstein and Chain 1940). It is active against some strains of staphylococci, and of intestinal streptococci of human origin. In high concentration it acts, though less energetically, on other pathogenic bacteria. It is natural to inquire whether it plays any part in the self-disinfecting action of the human skin. If so, it would seem that its rôle is relatively unimportant; since Colebrook (1930) records that many of the bacteria that are rapidly removed are not susceptible to its action, while *Micrococcus lysodeikticus* itself disappears less rapidly from the skin than such species as *Str. hæmolyticus*, *Bact. coli* or *Proteus vulgaris*.

The Mouth, Stomach and Intestinal Tract.—When bacteria gain access to the mouth, provided that they are not introduced as constituents of some food or drink that is immediately swallowed, they adhere to the mucus with which the lining epithelium is plentifully covered.

A series of careful studies by Bloomfield (1922*a*, *b*, *c*) have enabled him to trace in some detail their subsequent fate. In an initial series of experiments carbon particles were placed on the tongue, on the palate, in the sublingual space, on the mucosa of the cheek, on the posterior pharyngeal wall, or on the tonsil, and their subsequent movement was carefully observed. It was found that there was little or no tendency for the particles to spread at random over the surface of the mouth, but that they were removed from the various sites of implantation in an orderly manner. The general path of removal was directly backwards. There was no forward dissemination nor was there any tendency to wide lateral spread—carbon placed well to one side of the tongue near its tip did not, for instance, cross the mid-line. The paths of transit from the tongue, sublingual space and palate all converge at the base of the tongue. The tonsils, protected by the faucial pillars, occupy a position that is out of the way of these lines of passage so that under normal conditions particles that are deposited on the tongue are swept past them. The currents that bear the particles backwards are almost certainly the result of suction, set up by the lips, cheek, tongue and palate ; and particles are removed with particular rapidity from the sublingual space and from the lateral buccal mucosa, where the flow of saliva is maximal and the local formation of the mucosa is least favourable to retention. Particles placed directly on the tonsils are in part removed rapidly by the general flushing action of the saliva ; but those that once gain lodgment remain relatively stagnant for a considerable period of time. Having reached the base of the tongue the particles are periodically swallowed. In general, carbon particles are almost completely removed from the mouth within 15 to 30 minutes. Subsequent experiments with *Sarcina lutea* showed that bacteria introduced into the mouth were removed in the same fashion as carbon particles. Attempts were made to alter the flora of the tongue by thorough and repeated washing. It was found that the greater part of any bacteria artificially implanted could be rapidly removed in this way, but no amount of washing would produce any significant change in the character or number of the normal lingual flora. It would appear that bacteria entering the mouth are unable to persist on any of the mucous surfaces for more than a few hours unless their biological characters are such as to allow of successful colonization. Once colonized they become part of the local flora, and can no longer be dislodged by any simple mechanical means, nor probably by any ordinary disinfectant.

Human saliva is to some extent bactericidal. It is said to moderate the virulence of diphtheria bacilli given intradermally in guinea-pigs (Bézi 1932), and to be bactericidal for *C. diphtheriæ* (Dold and Weigmann 1934, Weigmann and Koehn 1936, Weigmann and Noeske 1937), and for a wide variety of cocci and intestinal pathogens (Dold *et al.* 1936). Dold and his colleagues gave the name " Inhibine " to the bactericidal principle of saliva, which they found to be thermolabile, and protein in nature. Casassa (1937) found the salivary principle to be heat-stable ; while Schäfer (1936) failed to confirm Dold's findings. (See also Auerswald 1938.)

Once bacteria have been swallowed they are subjected in the stomach and upper part of the duodenum to the action of the gastric juice, with its high hydrogen-ion concentration. It has long been recognized that the gastric secretion imposes a barrier to the passage of bacteria from the mouth to the intestine, but the careful studies of Arnold and his colleagues (Arnold 1926, 1927, 1928, 1929, Arnold and Brody 1926*a*, *b*) have placed our knowledge on a more detailed basis. By fixing various segments of the gastro-intestinal tract of the dog to the skin of the abdomen they were able to withdraw samples of the contents by sterile puncture as desired, and thus to determine the nature of the normal flora in relation to the acidity or alkalinity of the intestinal contents, and also the fate of other bacteria when administered by the mouth.

Table 66 shows the relation between the hydrogen-ion concentration of the intestinal contents and the prevailing bacterial flora at different levels.

TABLE 66

Portion of Small Intestine.	pH of Contents.	Bacterial Flora
Duodenum	5·2–6 0	Few Gram-positive cocci.
Jejunum, upper half . .	5·5–6·5	,, ,, ,, ,,
Jejunum, lower half . . .	6 0–7·0	Gram-positive cocci, few Gram-positive and Gram-negative bacilli.
Ileum	6 8–8·0	Rich and varied bacterial flora.

Using as test organisms *Chromobacterium prodigiosum* and *Ps. pyocyanea*, it was found that these bacteria, when introduced in watery suspensions into the empty stomach of a dog 12 to 18 hours after a meal, failed to reach the cæcum. Introduced in an alkaline buffered watery suspension or in alkaline buffered milk they reached the cæcum in large numbers. (See also Teale 1934 and Chapter 90).

Whether the bactericidal action of the duodenal contents is entirely determined by its hydrogen-ion concentration, is, perhaps, open to question (see Meyer and Löwenberg 1928).

Garrod (1937) found that gastric juice was markedly bactericidal for a number of organisms that infect by the mouth. *Br. abortus* was most susceptible; and after it, in diminishing order of susceptibility, *Br. melitensis*, *Salm. typhi*, *Salm. typhi-murium* and *Sh. flexneri*.

With regard to the possible effect of Fleming's lysozyme in the stomach and intestine, Goldsworthy and Florey (1930) have studied the distribution of this substance in mucus and mucosal extracts from several animal species, using as test organisms *Micrococcus lysodeikticus* and a series of unidentified susceptible bacteria isolated from the air of the laboratory. In the cat, no lysozyme could be detected in mucus from the stomach or colon, though small amounts could be demonstrated in saline extracts of the dried mucosæ. In the dog, detectable amounts were present in the mucosa of the intestinal tract, more in the colon than in the stomach. In the rabbit, the mucosal extracts were actively lytic. The colon was more active than the stomach, the small intestine falling between the two. In the guinea-pig, on the other hand, the mucosa from the stomach was more active than that from the intestine. What part this substance plays in eliminating susceptible species from the intestinal flora it is, however, impossible to say.

Quite apart, however, from the action of the contained lysozyme, there can be no doubt that the mucus itself provides a very efficient and important cleansing and protective mechanism. Florey (1933) has studied this mechanism in some detail. He finds that the mucus does not form a uniform coating over the epithelial cells of the mucosa, but is present in the form of a lace-like mesh-work spread over the mucosal surface. The villi free themselves from any small adherent particles by movements that bring the particles into contact with the mucus, to which they adhere. The mucus, with the adherent particles, is then rolled up into small masses by the intestinal movements, and is propelled onwards by the peristaltic movements of the bowel. Non-pathogenic bacteria, when introduced into the intestinal lumen, appear to behave very much as inert particles, except that those sensitive to lysozyme are dissolved when lysozyme is present. When a thick suspension of a pathogenic organism, such as *Salm. typhi-murium*, is introduced into an intestinal loop the resulting changes are very different. There is a considerable exudation of phagocytic cells into the gut lumen, and in places the epithelial cells desquamate, leaving bare the underlying stroma. Most of the mucosa, however, preserves its epithelial covering completely; and it would appear that the bacteria not infrequently enter, and pass through, the epithelial cells themselves.

It is of obvious interest to inquire whether those procedures that alter the normal conditions in such a way as to allow bacteria to pass alive through the

stomach and duodenum to the lower segments of the intestine have any effect in promoting their passage through the intestinal mucosa to the lymphatics and blood stream.

Arnold (1928) has studied this problem experimentally. *Bact. coli* and *Chromobacterium prodigiosum* were injected with minimal injury into a dog's duodenum, and counts were made of the bacteria in the lymph collected from a canula inserted into the thoracic duct. The results are summarized in Table 67. It will be noted that alkalinization alone does not suffice to induce the transit of bacteria from the intestinal lumen to the lymphatics, but that addition of bile, or still better of egg white, so alters the conditions at the mucosal surface that such passage occurs. The negative findings with the other suspending fluids employed are in accord with those of Neisser (1896) and of Teale and Embleton (1914). It would appear that only under quite exceptional conditions is the intestinal mucosa permeable to living bacteria; and it would seem (Nedzel and Arnold 1931) that raw egg albumin is peculiarly potent in inducing the passage of bacteria not only from the intestinal lumen to the lymphatics and the blood, but in the reverse direction from the blood stream to the intestinal lumen.

TABLE 67

Solution injected with Bacteria.	Average No. of Bacteria in Lymph per ml.	Time of Appearance after Injections
Alkaline solution and egg white. .	500 to 1,000	First 5 mins. and lasting for 30 mins.
Neutral solution and egg white . .	None	—
Alkaline solution	None	—
Neutral solution	None	—
Alkaline solution and bile . . .	50 to 100	First 5 mins. and lasting for 20 mins.
Neutral solution and bile. . . .	3 to 5	During first 30 mins.
Alkaline solution and dog's serum .	None	—
Neutral solution and dog's serum .	None	—

The Nose, Nasopharynx and Respiratory Tract.—The nasal cavities are in part protected from air-borne bacteria by the anatomy of the anterior nares. The greater part of the inhaled bacteria appear to be arrested at or near the nasal orifices (see Thomson and Hewlett 1896). Those that pass beyond this point adhere to the film of mucus that covers the nasal mucosa, and are then swept back—in this case by the current set up by the ciliated epithelium—towards the naso- and oro-pharnyx, where they join the bacteria being swept back by suction currents from the mouth (see Bloomfield 1919, 1922*d*). Droplets and droplet nuclei produced by spraying a culture of *Chr. prodigiosum* into air, in numbers up to 700 per litre, were found by Rooks (1939) to be filtered out in the nasal passages with an efficiency of 87·5 per cent. The efficiency was less—62·0 per cent. —when air containing only droplet nuclei was tested.

Whether mechanical clearance is the only factor concerned is again uncertain. Arnold, Ostrom and Singer (1928) introduced *Chromobacterium prodigiosum* and *Bact. coli.* by spraying, into the nose of men, rabbits, dogs and guinea-pigs, and noted their rapid disappearance. They state that swabs taken from the posterior pharyngeal wall gave no indication that there was any extensive passage of the bacteria to the throat; but they were unable to detect in nasal washings or secretions any substance that was bactericidal to the test organisms employed. It may be noted that the rate of disappearance recorded by Arnold and his colleagues—a diminution in numbers during the first 5 minutes, and total disappearance within half an hour—was more rapid than that recorded by Bloomfield—

little change in 2 hours but almost complete disappearance in 24. This slower rate of elimination was also noted by Bloomfield in the case of particles of kieselguhr placed on the nasal septum. The route followed by substances introduced into the nose to some extent depends on the animal tested, the nature of the substance and the method of its administration. Rake (1937), for example, records a ready intercellular penetration of the olfactory mucosa of mice by pneumococci, *Salm. enteritidis*, particles of Prussian blue and the virus of equine encephalomyelitis, but only slow penetration by rabies and certain other viruses; while in rabbits, virulent pneumococci penetrate the nasal mucosa only if the containing fluids are kept in prolonged contact with it (Cannon and Walsh 1937).

Lysozyme is known to be present in high concentration in the nasal secretions, and it is probable that it plays a part in freeing the nasal cavities from those species of bacteria against which it is active. A substance resembling Dold's Inhibine in bactericidal power has been described in the nasal secretion of normal persons (Ignatius 1936). Burnet, Lush and Jackson (1939) found a heat-labile, possibly enzymic, substance in nasal secretion that inactivated certain viruses, notably those of influenza, herpes and poliomyelitis. Vaccinia virus was insusceptible to its action.

Whatever may be the nature of the complex of mechanisms involved there is every reason to suppose that the same result is attained in the nose as in the mouth. The majority of bacteria that enter at the external nares are removed or destroyed, and fail to obtain any but the most transient footing among the normal nasal flora; those that survive and multiply do so because they are in some way adapted for successful colonization, and once such colonization has occurred they may persist for an indefinite period of time.

The conditions in the nasopharynx have been sufficiently dealt with in considering the elimination of bacteria from the mouth.

It would appear (Calamida and Bartarelli 1902) that the accessory nasal sinuses are normally sterile.

There is little doubt that in health the trachea and bronchi contain few if any living bacteria (see Thomson and Hewlett 1896, Bloomfield 1922*d*); and it is probable that the main protective mechanism consists in the filtering action of the nasal passages combined with the adherence of bacteria which pass the posterior nares, or are drawn in *via* the mouth, to the pharyngeal or upper laryngeal mucosa. It is, however, quite certain, from the ordinary experience of the *post-mortem* room, that dust particles, and hence presumably bacteria, reach the lungs in numbers that, if minimal in any short period of time, attain a considerable total in the course of months or years.

Under certain conditions, mainly experimental (see Nenninger 1901, Paul 1902, Quesnil 1902, Wherry and Butterfield 1920, Bloomfield 1922*d*, Trillat 1923, Stillman and Branch 1926), bacteria inspired as dust or droplet nuclei may reach the peripheral alveoli of the lung in relatively large numbers. Jones (1922) records that the lungs of normal herbivorous laboratory animals contain bacilli derived from the dust present in their dry food-stuffs. It appears, however, that those which penetrate so deeply never form more than a small fraction of the total number inspired. Cralley (1942) has shown that when droplet nuclei made from cultures of *Chromobacterium prodigiosum* are inhaled by the rabbit more than 96 per cent. are retained in the upper respiratory tract. Of the bacteria contained in those nuclei that reach the lung alveoli, 90 per cent. disappear in 3 hours, and none can be found after 12 hours. Once past the vocal cords, the inspired organisms are probably dealt with in three ways. They may adhere to mucus and be expec-

torated; they may be driven upwards by the ciliated epithelium (Florey, Carleton and Wells 1932, Barclay and Franklin 1937) which extends as far down as the smaller bronchioles; or they may reach the alveoli, and be ingested by phagocytic cells, and removed *via* the lymphatic plexus to the regional lymph nodes. It is probable that ingestion by the alveolar macrophages is in some cases followed by intracellular digestion of the bacteria *in situ*. There is little evidence of an immediate bactericidal mechanism in the lungs, though Dold (1943) found that cultures of *B. anthracis* and *Chr. prodigiosum* were rapidly killed when applied to the inner surface of the partly excised trachea of the guinea-pig. From the fact that autoclaving of the tissue destroyed its bactericidal power, he inferred the presence of an "inhibine" similar to that reported in saliva.

Fluid that contains bacteria, once it is past the epiglottis, flows readily to the terminal parts of the lung, the distribution depending to some extent upon the posture of the animal. Infection of the lung is more easily achieved by instillation of fluid than by inspiration of bacterial dusts or mists (see Webster and Clow 1933, Cannon and Walsh 1937).

The Conjunctivæ.—It is convenient to consider the conjunctivæ in connection with the upper respiratory tract. The mechanical flushing action of the tears is an obvious phenomenon, and there is little doubt that the main route of removal is *via* the lachrymal duct.

Maxcy (1919) was able to recover *Chromobacterium prodigiosum* from the nose within 5 minutes after its introduction into the conjunctival sac. Stort (1891) failed to recover *Bact. coli* from the rabbit's conjunctiva 1 hour after its implantation; but found that it persisted for a much longer time if the lachrymal duct were previously tied.

Tears have the highest lysozyme content of any secretion yet examined. At a dilution of 1 : 40,000 they may cause complete lysis of a suspension of *Micrococcus lysodeikticus*. There can be little doubt that this agent suffices to rid the conjunctiva of many of the organisms that come in contact with it, and that it plays a part in the local defence of this region against certain pathogenic bacteria (see Ridley 1928).

The Genital Tract.—It is probable, though there is no definite evidence on the point, that the external genitals, so far as they are covered with a complete dermal layer, share the bactericidal action of the skin elsewhere. There are probably additional selective factors—favourable or inhibitory for particular bacterial species—resulting from localized glandular secretions, or from differences in the amount of moisture in the skin.

The urethra in the male and female is normally sterile, or contains in the neighbourhood of the meatus a few staphylococci and diphtheroid bacilli. The mechanical flushing action of the urine, combined perhaps with its slightly acid reaction (pH 6·0), is probably an important factor. Dold and Deck (1941) have described a heat-stable type of inhibine in fresh urine, active against a wide variety of pathogenic species.

The vagina has a highly characteristic flora, consisting largely of aciduric bacilli of the Döderlein type (see Küster 1929, Schultheiss 1929). The normal vaginal secretion has a marked bactericidal action on many species of bacteria. Thus Menge (1894) found that *Ps. pyocyanea*, staphylococci and streptococci introduced experimentally into the vagina could not be recovered after 21 to 26 hours (see also p. 1210).

Bacterial substances have been described in cow's milk, active against cocci and coliform bacilli (see Hobbs 1939, Morris 1943, 1945, Green and Pauli 1943). It is possible that in man such substances may contribute to the defence of the lactating mammary gland against certain infections.

The Efficiency of the Bactericidal or Inhibitory Mechanisms Operating on Body Surfaces.

It is extremely difficult to assess the relative protective value of the factors that we have described above, as compared with the mechanisms that we shall discuss in subsequent chapters. It is obvious that they often fail; but how frequently they succeed we cannot tell.

That they are of real importance can easily be shown experimentally by noting the different doses of bacteria that are required to induce experimental infection by different routes.

To take a single example from many available, the dose of *Salm. typhi-murium* that will, on the average, lead to a fatal infection when injected intraperitoneally into a mouse of 18–22 gm. is somewhere between 1,000 and 1,000,000 times smaller than the dose that will lead to the same result when administered by the mouth. A series of observed figures for groups of 85 mice per dose (intraperitoneal injection) and of 75 mice per dose (*per os* infection) are given in Table 68.

TABLE 68

SHOWING THE PERCENTAGE MORTALITIES OBSERVED IN GROUPS OF MICE AFTER THE ADMINISTRATION OF VARIOUS DOSES OF A VIRULENT STRAIN OF *Salm. typhi-murium* INTRAPERITONEALLY OR *per os*.

Number of Bacilli administered.	Mortality per cent.	
	Intraperitoneal.	*Per os.*
1,000,000,000	Not tested	48·75
10,000,000	98·8	28·25
100,000	84·7	22·50
1,000	65·9	15·00
10	28·2	Not tested

SUMMARY

There are a series of mechanisms, concerning the exact nature of which we as yet know relatively little, that tend toward the mechanical removal or local destruction of most of those bacteria that reach the various body surfaces from the outside world. These mechanisms vary in nature from one locality to another; each body surface—the skin, the conjunctivæ, the mucous membranes of the nose, mouth, pharynx, intestinal tract, genital tract and so on—tends to develop and maintain its own characteristic bacterial flora, the flora of any one locality differing both qualitatively and quantitatively from that of another, and the flora of a particular locality (say the nasopharynx) showing minor but significant differences as we pass from one person to another, or study a single person over a prolonged period of time.

The resistance thus afforded to colonization of bacterial immigrants is certainly an important factor in the body's first line of defence, but its effectiveness in relation to any particular natural infection, or under any specified set of conditions, cannot at present be estimated with any accuracy. We know only that this barrier is, in fact, often penetrated, and that the fate of the host then depends on the mechanisms of antibacterial or antitoxic immunity that we shall consider in subsequent chapters.

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CHAPTER 46

THE MECHANISMS OF ANTITOXIC IMMUNITY

For our immediate purpose this type of immunity may be dealt with very briefly.

Apart from certain cases of natural insusceptibility, such as the insusceptibility of birds and of cold-blooded animals to tetanus toxin (Vaillard 1892), immunity to bacterial exotoxins depends on the presence in the circulating blood of antitoxins which exert a specific neutralizing action. These antitoxins may have been naturally acquired, as the result of past infections, overt or latent ; or they may have been produced in response to purposeful immunization with modified or unmodified toxin. They can be passively transferred from one animal to another—naturally from mother to offspring *via* the placental vessels or in the colostrum, artificially by injecting an antitoxic serum into a susceptible host.

The original observations on this type of immunity were made by von Behring and Kitasato (1890), who showed that the sera of animals that had received repeated injections of non-lethal doses of tetanus toxin or of diphtheria toxin had acquired the property of specifically neutralizing these toxins and thus preventing their poisonous effects.

Since that time a number of antitoxic sera have been prepared, and applied in prophylaxis or treatment. We are here concerned only with anti-exotoxins—in the sense in which these were defined in Chapter 44. Among these we should include —in addition to diphtheria and tetanus antitoxins—the antitoxins that neutralize the erythrogenic toxin produced by the hæmolytic streptococcus and the toxins of the *Staphylococcus aureus*, of Shiga's dysentery bacillus, and of a variety of pathogenic anaerobes, such as *Cl. welchii*, *Cl. septicum*, *Cl. œdematiens* and the lamb-dysentery bacillus. The antitoxin which neutralizes the toxin of *Cl. botulinum* is, of course, a typical anti-exotoxin ; but, as we have explained above, it is doubtful whether *Cl. botulinum* should be included among the pathogenic bacteria in the ordinary sense, since there is little evidence that it is capable of giving rise to infection *in vivo*.

A characteristic of antitoxic immunity is the relatively low concentration of antitoxin in the circulating blood which suffices to afford protection against natural infection, provided that it has been produced as the result of active immunization. In the particular case of diphtheria in man, we have good reasons for believing that less than 0·01 unit of antitoxin per ml. of blood serum will render a person immune to the natural disease (see Glenny 1925). Under such conditions the initial toxin produced is neutralized as it passes from the site of infection before it reaches the susceptible cells. But it is clear that this small amount of circulating antitoxin will not suffice for long, and that more will be needed to neutralize additional toxin as and when it is produced. An actively immune person or animal

differs from a non-immune not only in possessing circulating antitoxin but in the readiness and rapidity with which more antitoxin is produced in response to the stimulus provided by the access of toxin to the tissues (see Chapter 50). The equilibrium between the rate of toxin-production by the bacterium and the rate of antitoxin-production by the host is, in the immune animal, readjusted to the host's advantage. A degree of antitoxic immunity that afforded ample protection against infection with a toxigenic organism could of course be broken down by the inoculation, in a single dose, of an overwhelming amount of toxin.

Although the efficacy of antitoxic immunity is, in the main, determined by quantitative relationships of this kind, there can be little doubt that qualitative differences between one antitoxin and another affect the firmness of toxin-antitoxin union, and so the degree of protection afforded.

It was pointed out many years ago that the curative effect of antidiphtheria sera, as judged by experiments on laboratory animals, did not always run parallel to the antitoxin content as assessed by the ordinary technique (see, for instance, Kraus and Schwoner 1908, Kraus and Baecher 1913). Kraus and his colleagues suggested that one important quality of an antitoxic serum, not measured or expressed in the official standardization in terms of Antitoxin Units, was the character that determined the *rate* of neutralization, and for this character they proposed the name *avidity*. The conception of avidity has been considerably modified, and rendered far more precise, by later observations recorded by Madsen and Schmidt and by Glenny and his colleagues.

Madsen and Schmidt (1929) found that, with some antitoxic sera, toxin-antitoxin mixtures that were neutral as judged by subcutaneous injection in the guinea-pig might produce a fatal toxæmia when injected intravenously into rabbits. Glenny and his colleagues (see Glenny and Barr 1932*a*, *b*, Glenny *et al.* 1932) note that, with sera of this kind, it is possible to prepare mixtures such that rabbits will survive the intravenous injection of 10 ml. but not of amounts varying from 0·001 ml. to 0·5 ml. The greater dilution of the small inocula in the rabbit's circulating blood apparently results in the dissociation of a lethal dose of toxin. Glenny and his colleagues have devised a method for estimating the avidity of antitoxic serum in terms of a *dilution-ratio*, which they define as the ratio of the amount of antitoxin necessary to form a neutral mixture with one L_r dose of toxin in a total volume of 2 ml., to the amount necessary to form a neutral mixture with the same amount of toxin in a total volume of 200 ml., neutrality being determined in each case by the injection of 0·2 ml. of the mixture into the skin of a guinea-pig. Although Glenny and Barr, in agreement with Madsen and Schmidt, note that avid sera commonly give rapid flocculation when examined by Ramon's method, they regard firmness of union, rather than rapidity of reaction, as the essential character in avidity. They note that the avidity of antitoxic sera appears to be determined, at least in part, by the type of serum protein with which the antitoxin is associated. Thus (Barr and Glenny 1931*a*, *b*, Glenny and Barr 1932*a*, Glenny *et al.* 1932), if successive globulin fractions are precipitated with increasing amounts of ammonium sulphate, the earlier fractions are more avid than the later. Avidity is sometimes a property of a particular electrophoretic fraction of an antiserum (see Chapter 7). According to Kekwick and his colleagues (1941) the γ-globulin of diphtheria antitoxic serum is more avid, flocculates more rapidly *in vitro*, and has a greater combining power than the α-globulin (see also McSweeney 1941). These phenomena are by no means confined to the particular case of diphtheria toxin and antitoxin. It would seem indeed that non-avid sera are more frequently met with among antitoxic sera of other types (Glenny *et al.* 1932). (See also Chapters 47 and 55 for analogous observations in the case of antibacterial and antiviral sera.)

The increased resistance afforded by specific antitoxic immunity is determined almost entirely by the interception and neutralization of the toxin before it reaches

the susceptible cells. Once these have been attacked, the antitoxin is relatively ineffective.

Glenny and Hopkins (1925) have studied the effect of the administration of various doses of antitoxin at different times after the intradermal injection of a Schick dose of diphtheria toxin into a guinea-pig. This amount of toxin produces a characteristic local reaction at the site of inoculation; 0·001 unit of antitoxin is sufficient to prevent this reaction if the toxin and antitoxin are mixed together before inoculation. If, however, the toxin is injected before the antitoxin, neither 10 units of antitoxin injected intravenously 15 minutes later, nor 1,000 units injected intravenously 30 minutes later, are sufficient to prevent the appearance of a small reaction at the site of the injection of the toxin. The size of the reaction is, however, very greatly reduced as compared with that which occurs when no antitoxin is given. If 10 units of antitoxin are administered intravenously 30 minutes after the intradermal injection of the toxin, or 1,000 units intravenously after 1½ hours, or intramuscularly after 45 minutes, the size of the lesion is reduced to about half its usual diameter. A slight reduction in the size of the reaction is obtained if 1,000 units are injected intravenously after 2½ hours, or intramuscularly after 1¾ hours; but the subcutaneous injection of this amount of antitoxin 15 minutes after the intradermal inoculation of toxin leads to no reduction in the size of the reaction. We may note the relatively enormous dose of antitoxin that is required to neutralize the effect of the toxin under the conditions of this experiment; 1,000 units of antitoxin are equivalent to 1,000,000 times the dose of toxin injected into the skin, yet this amount administered intravenously 30 minutes after the intradermal injection fails to suppress completely the local toxic effect. Friedemann and Zuger (1939) record analogous results in rabbits (see p. 1060).

From a consideration of the amounts of intravenous antitoxin required to protect dogs from locally injected tetanus toxin, Abel and Chalian (1938) concluded that the protective dose increased exponentially with increasing interval between injection of toxin and antitoxin. Similar conclusions were reached by Miles and Miles (1943), who found that mice receiving an intramuscular dose of *Cl. welchii* toxin that would kill them in 6–8 hours could be protected by an intravenous dose of 0·1 unit of antitoxin after 1 hour; after 4 hours an intravenous dose of 40 units was ineffective. Stockhusen (1936), however, records that diphtheria toxin adsorbed to washed guinea-pig organs can to some extent be dissociated from the cells by a dilute solution of antitoxin, indicating that there are various degrees of firmness in the fixation of toxin to tissues (see also Chapter 77).

The explanation of the disproportionately large doses of antitoxin required for the neutralization of established intoxications does not lie wholly in the adsorption of toxin on to susceptible tissues in such a way that antitoxin cannot neutralize it. We shall discuss in Chapter 47 the influence of the anatomical and physiological peculiarities of the site of infection on this phenomenon.

A purely antitoxic immunity affords adequate protection against such a disease as diphtheria, in which the tissue invasion is minimal and the ill effects are due almost entirely to the toxæmia. Does it afford effective protection against those bacteria that are invasive as well as toxigenic? We should expect that it would protect only against that part of the total pathological effect that is due to the action of the toxin itself. If the toxin plays any important part in the invasive process, the antitoxin might to that extent lessen the extent of infection. But we should expect modification of the infection rather than complete immunity. The available evidence is in accord with our expectations.

The presence of circulating antitoxin, active against the rash-producing toxin of the hæmolytic streptococcus, affords protection against clinical scarlet fever, that is, against the obvious effects of the toxin itself; but it does not appear to protect against the local lesion in the throat—the acute tonsillitis (Okell 1932). Again, it would seem (Burt-White,

Colebrook and others 1930, Baird and Cruickshank 1930, Stent 1930) that the presence of circulating antitoxin, as revealed by a negative Dick test in pregnant women during the later stages of gestation (see p. 1481) affords little if any protection against a severe or fatal infection with hæmolytic streptococci during the puerperium. These clinical findings are borne out by the results of experiment. Thus, Parish and Okell (1927) found that a potent antitoxic serum, which would protect rabbits against acute toxæmic death following the intravenous injection of large doses of living cultures of hæmolytic streptococci, did not prevent a fatal infection, leading to death after a week or more, associated with multiple foci of infection. Nevertheless, as reference to Chapter 66 will show, in patients with scarlet fever, antitoxic sera sometimes appear to influence the course, not only of the toxæmia, but of complications directly referable to the bacterial invasion by the *Str. pyogenes*.

Closely analogous results have been obtained in experimental staphylococcal infections in the rabbit (Burnet 1929, Burnet and Kellaway 1930, Kellaway, Burnet and Williams 1930). The potent staphylococcal toxin is neutralized, in multiple proportions, by the specific antitoxin; and a rabbit can be actively or passively immunized against the poisonous effect of a toxin-containing filtrate. But such immunized animals are still susceptible to the injection of living staphylococci and succumb to a pyæmic infection, though they survive rather longer than non-immunized controls. Later studies, however, indicate clearly that in rabbits antitoxin protects, though not completely, against bacterial invasion. Ramon and his colleagues (1936) found that intravenous virulent *Staph. aureus* did not kill rabbits with a high level of antitoxin in the serum, whereas rabbits with a low level succumbed. Kitching and Farrell (1936) observed that immunization with toxoid protected rabbits against infection, but that immunization with bacterial bodies, though it produced abundant agglutinins, was ineffective. Both Forssman (1936) and Smith (1937) demonstrated that active immunization with staphylococcal products was effective with those antigens that induced the formation of antitoxic antibodies. Downie (1937) studied active and passive immunization against living staphylococci and staphylococcal toxin, intradermally administered, and concluded that as far as rabbits were concerned, antitoxic antibodies were more beneficial than antibacterial in immunity to the bacterial infection (see also Mercier 1937, Delaunay 1938).

To speak generally, it is clear that antitoxin, by removing the burden of progressive intoxication from the tissues of the animal, may permit them to exert their antibacterial action with greater efficiency. Thus Ritossa (1937) found that human leucocytes readily ingested virulent diphtheria bacilli, but that many of them subsequently degenerated. The degeneration could be prevented, and phagocytosis improved, if antitoxin was added to the bacilli before phagocytosis. Stewart (1943) observed a similar phenomenon in guinea-pigs injected intraperitoneally with virulent *Cl. welchii*. In animals actively or passively immunized with antitoxin, there was marked intraperitoneal phagocytosis; in non-immune animals, there was little phagocytosis, though leucocytes were abundant, and the animals died of the infection.

Thus, we may say that antitoxic immunity is highly effective *so far as it goes* —much more effective, within its own sphere, than the specific antibacterial immunity discussed in the next chapter; for it is direct in its action, does not depend on the efficient functioning of a complex system of phagocytic cells, and is complete and final in its effect on the noxious agent. If the invasive power of an infecting toxigenic organism is so low that the normal antibacterial defence mechanism can be relied on to suppress it, or to hold it in check, an added antitoxic immunity will afford almost complete protection against the corresponding disease.

It will be observed that the characteristic mechanisms of antitoxic immunity, as here defined, are brought into play only against those toxins that diffuse freely from a locus of infection. Reactions between endotoxins and the corresponding

antibodies undoubtedly play a part, perhaps a large part, in infection and resistance; but only in a few instances is it possible to differentiate clearly between anti-endotoxic and antibacterial immunity. It has, for instance, been noted in Chapter 44 that some endotoxins are identical with the surface somatic antigens that determine the immunological reactions of the intact bacterial cells.

This chapter is itself a summary.

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CHAPTER 47

THE MECHANISMS CONCERNED IN SPECIFIC ANTIBACTERIAL IMMUNITY

THE only sound basis for a discussion of this subject would be to treat it as a problem in comparative pathology, describing in turn the reaction to infection of unicellular organisms, of simple multicellular animals, of the invertebrates, and of the vertebrates, culminating in the mammals and man. It happens that, owing to the classical studies of Metchnikoff, our earlier knowledge of the cellular reactions involved in immunity was developed largely along these lines; and any serious student of the subject should make himself familiar with the work of Metchnikoff and his school (Metchnikoff 1901). For a review of Immunity in Invertebrates, see Huff (1940). For our present purpose, however, it will suffice to note that the mechanisms that are described in this chapter are clearly traceable, in their gradually increasing complexity, differentiation and modification, from the simplest to the most highly organized of animal hosts. In the pages immediately following we shall be concerned almost entirely with happenings in the small mammals of the laboratory; and it will be convenient to deal first with the reactions of normal animals, and then to consider how these reactions are modified by active or passive immunization.

The Reactions which follow the Intravenous Injection of Dyes or Suspensions of Inert Particles into Normal Animals.

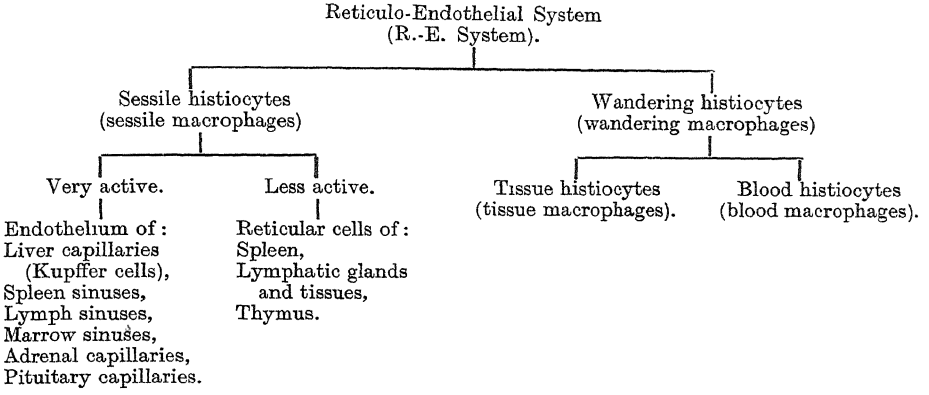
The simplest introduction to a discussion of the mechanisms concerned in antibacterial immunity is a brief description of the way in which the living tissues deal with certain dyes and suspensions.

In pre-bacteriological days Hoffmann and von Recklinghausen (1867) and Ponfick (1869) noted that particles of carmine and vermilion, when injected intravenously into animals, were not eliminated in the urine or the bile, but were deposited in various organs such as the spleen, liver, lymph nodes and bone-marrow, where they remained recognizable for weeks. More recently, particularly within the last two decades, extensive and detailed studies have been carried out on the effects of intravital staining with a variety of dyes such as trypan blue, vital red, pyrrhol blue (isamine blue), diaminefast scarlet and benzopurpurin, or with suspensions of finely divided carbon (Indian ink) or colloidal ferric oxide (saccharated oxide of iron). An account of an investigation along these lines and a good review of the literature will be found in a series of papers by Cappell (1929, 1930).

Briefly, these studies have shown that the various dyes are stored, in the form of granules or in intracellular vacuoles, by certain cells that have a characteristic distribution throughout the body. The histologist calls these cells *histiocytes*, the immunologist knows them best by Metchnikoff's name, *macrophages*. The most active may be divided into two main types, the sessile and the wandering. The

most active of the sessile histiocytes are those found in certain specialized areas of the vascular or lymphatic endothelium—the endothelium of the liver capillaries (Kupffer cells), of the spleen sinuses, of the venous sinusoids of the bone-marrow, of the capillaries and medullary sinusoids of the adrenals, of the capillaries of the pituitary gland, and of the sinuses of the lymph glands throughout the body. Somewhat less active in this respect are the reticulocytes, which do not form a lining to blood or lymphatic channels, but are disposed about the reticulum fibres in the interstices of the tissues. The wandering histiocytes are found throughout the tissue spaces, and some of them find their way into the circulating blood, particularly into the vessels of the internal organs.

The common character of dye-storage possessed by cells of this type has led many observers, and particularly Aschoff (1924), to regard them as an integrated system of cells fulfilling a particular bodily function. Aschoff has coined for them the generic term of *reticulo-endothelial system* (R.-E. system, for short). This system, as described by him, is summarized in schematic form as follows.



The schema classifies the various cells belonging to the system, and does not necessarily represent a genealogy of the cells found in the animal body. The phylogenetic interrelations of the various types of phagocytic cell in the mammalian body is still in a controversial stage. For our purpose the R.-E. system is a functional unit consisting of cells derived from the mesenchyme, having in common a phagocytic activity (see, for example, Doan 1940); and we are particularly concerned with the more active phagocytic cells, and the sessile histiocytes of the lymph glands, liver, spleen and bone-marrow, and the wandering histiocytes in general.

As Cappell emphasizes, there is no gradual transition from the less specialized endothelium to that in which the sessile histiocytes are found. The endothelium of the lymph sinuses abruptly assumes a pronounced vital-staining capacity, in marked contrast to that of the endothelium of the afferent and efferent lymphatics; while the same contrast exists between the endothelium of the spleen sinuses, liver capillaries, etc., and the vascular endothelium in general. There is probably some degree of functional differentiation between different parts of the reticulo-endothelial system. Some histiocyte depôts may be more active in removing effete blood corpuscles or leucocytes, others in removing bacteria or smaller particles. There is also a difference between one animal species and another in the relative activity of the various depôts of sessile histiocytes. Thus the bone-marrow of the mouse is far less active than the bone-marrow of the rabbit (Cappell 1930). In birds, the mass of the reticulo-endothelial system is concentrated in the liver.

The histiocytes are not, however, the only phagocytic cells with which we are concerned. The polymorphonuclear leucocytes of the blood, although they do not take up vital stains in the same way as the cells of the reticulo-endothelial system, play an active part in removing particulate material from the circulation under certain specialized conditions.

The intervention of these cells is, for instance, brought about by the injection, directly into the blood stream, of an Indian ink suspension or of some similar material. Following such an inoculation, the polymorphonuclear cells accumulate in certain areas—most abundantly in the pulmonary capillaries, to a less extent in the veins and sinusoids of the liver, spleen and other viscera. The abundance of polymorphonuclear cells in the lungs, which may be associated with a definite leucopenia in the peripheral circulation, is so characteristic a feature of the reaction as to make it quite clear that some mechanism is at work which temporarily retains the circulating leucocytes in this particular situation. While in the lungs the polymorphonuclear cells take up a certain number of the injected carbon particles; but the degree of phagocytosis is relatively slight, and in marked contrast to the active engorgement of particles by the histiocytes of the reticulo-endothelial system (Dudgeon and Goadby 1931). In the course of a few hours the majority of the polymorphonuclear cells leave the lung capillaries. Some of them pass into the alveolar spaces, some along the lymphatics to the regional lymph glands; but the great majority appear to make their way back into the general circulation and so pass to the spleen sinuses, where they appear to be taken up by macrophages.

There is still another mechanism at work in freeing the general circulation from injected foreign particles, in which no cellular elements are primarily concerned. Many suspensions, including carmine and Indian ink, are unstable when introduced into the blood stream. The dispersed particles are rapidly agglutinated into larger masses, and these appear to be filtered off from the circulating blood, during its passage through the finer capillaries. These aggregates are themselves phagocytosed at later stages. It has been noted that the aggregates of carbon particles contain large numbers of blood-platelets (Delrez and Govaerts 1918), and their formation is associated with a definite peripheral thrombopenia (Dudgeon and Goadby 1931). Whether the platelets play any active part in the process of flocculation is, however, very doubtful. The phenomenon of aggregation—certainly in the case of bacteria and presumably in that of any particulate material—appears to be unaffected after the elimination of platelets by the injection of an antiplatelet serum (Govaerts 1921*a*, *b*, Bull and McKee 1922).

Confining ourselves for the moment to the particular case of the direct introduction of particulate matter into the blood stream, we may inquire in what ways our description needs modification or extension when our injected particles are living bacterial cells.

The Reactions which follow the Intravenous Injection of Bacteria into Normal Animals.

There is no doubt at all that the mechanism brought into play in clearing the blood stream from bacterial cells is in the main identical with that which frees it from inert particles of the same order of size (Wyssokowitch 1886, Werigo 1894, Opitz 1898, Bardach 1889, Métin 1900, Bail 1905, Bull 1914–16, Kyes 1916, Bartlett and Ozaki 1917, 1918, Wright 1927 and many others). The reticulo-endothelial cells, particularly those of the liver, spleen and bone-marrow, actively phagocytose the injected bacteria. The mechanism of aggregation, followed by the removal of the bacterial aggregates from the general circulation by their retention in the

lung capillaries, is sometimes very prominent (Bull 1914–16, Bartlett and Ozaki 1917, 1918, Wright 1927, Dudgeon and Goadby 1931). This is accompanied, as in the case of the intravenous injection of carbon particles, by an accumulation of polymorphonuclear cells in the lung capillaries, associated with a temporary peripheral leucopenia (see Levaditi 1901, Andrewes 1910). But the polymorphonuclear cells appear to play a more active part in phagocytosing bacteria than in phagocytosing carbon particles (Dudgeon and Goadby 1931). It is probable that these cells with their ingested bacteria are subsequently carried to the reticulo-endothelial depôts in the spleen, liver and elsewhere, and are there phagocytosed by the sessile histiocytes. Such wandering histiocytes as are present in the lung capillaries ingest the bacterial cells, or cell-aggregates, directly.

So far then as we can regard bacteria merely as foreign particles, our description of the reaction of the tissues to the intravenous injection of vital stains or carbon particles needs little modification. But, in fact, the bacteria we inject are alive and capable of multiplication; and it is the balance between their capacity to multiply and the capacity of the tissues to remove them that determines the fate of the animal host.

Bull (1914–16) counted the viable bacteria in the systemic blood stream of dogs and rabbits at various intervals after the intravenous inoculation of a bacterial suspension. Using pneumococci and streptococci he found that there was at first a sharp and progressive fall in the number of organisms in the circulating blood, lasting until about the 5th hour. After this there was in most cases a secondary rise, varying in degree and persistence with the virulence of the organism and the resistance of the host. With strains of low virulence the numbers then declined until the blood became sterile. Typhoid bacilli, injected directly into the blood stream, showed a similar rapid fall in numbers within a few minutes of inoculation. In one experiment they fell from 10,000,000 per ml. 1 minute after injection to 40 per ml. at the end of 15 minutes.

Wright (1927) has carried out a very detailed series of experiments on experimental pneumococcal septicæmia in the rabbit, and the following illustrative examples are taken from his paper.

TABLE 69

LIVING PNEUMOCOCCI PER ML. OF CIRCULATING BLOOD AT STATED TIMES AFTER INOCULATION OF AVIRULENT, SLIGHTLY VIRULENT, AND HIGHLY VIRULENT PNEUMOCOCCI INTO NORMAL RABBITS.

Time.	Avirulent.	Slightly Virulent.	Highly Virulent.
Immediately	8,900,000	1,030,000	1,070,000
2 hours	206	20,800	137,000
5 ,,	2	340	25,000
24 ,,	0	1,300	1,510,000
48 ,,	—	134	Dead
96 ,,	—	0	—

In Table 69 are set out the numbers of living pneumococci per ml. of circulating blood at varying intervals after the intravenous injection of (*a*) an avirulent, (*b*) a slightly virulent, and (*c*) a highly virulent strain. In Fig. 240 the same series of observations are set out in chart form, the logarithms of the numbers of pneumococci per ml. of circulating blood being plotted as ordinates against time as abscissæ. The figures need no comment. With an avirulent strain the clearance of the organisms from the blood stream is rapid and permanent. With a slightly virulent strain there is the same initial rapid clearance, so that

99·97 per cent. of the injected organisms have been removed from the blood stream by the end of the 5th hour. There is then a period during which the capacity of this strain of pneumococcus to multiply in the tissues—limited though it is—makes itself felt, and the number of viable organisms rises from 340 per ml. at the 5th hour to 1,300 per ml. at the 24th hour. But the clearing mechanism is now removing the newly generated bacterial cells at a greater rate than this particular strain can produce them. The number of organisms per ml. sinks to 134 at the 48th hour, and the blood is sterile by the 96th. The

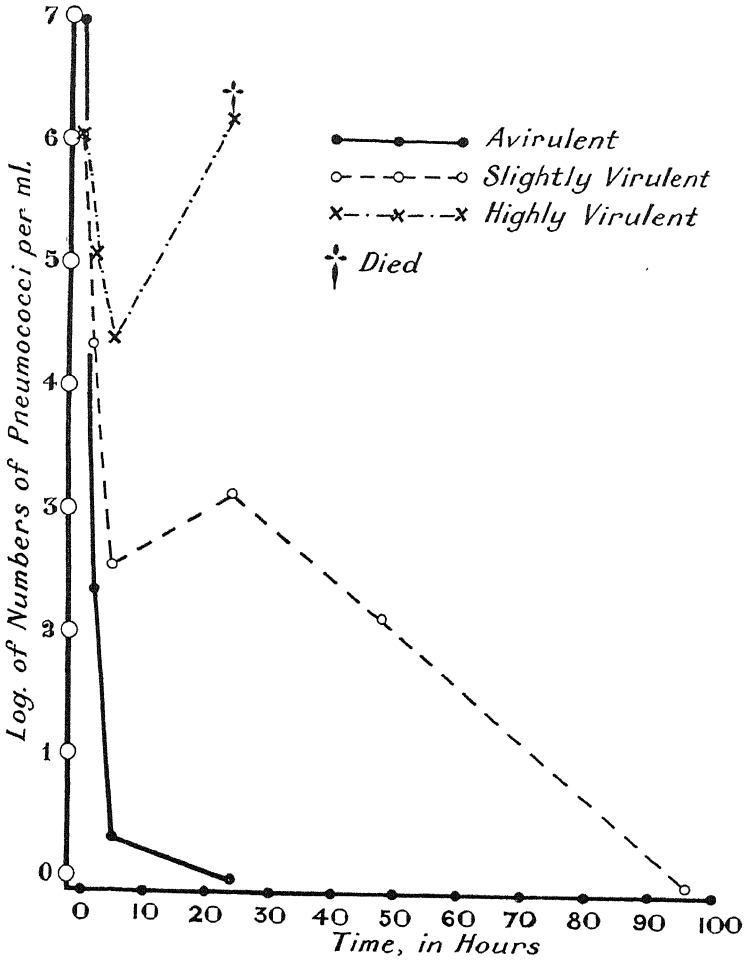


FIG. 240.

highly virulent strain is not proof against the initial action of the clearing mechanism. By the 5th hour 97·7 per cent. of the injected pneumococci have been removed from the blood stream. But when the parasite's capacity for multiplication is brought fully into play it overwhelms the defence mechanism of the host. By the 24th hour the number of pneumococci per ml. of circulating blood has risen to 1,510,000; and between the 24th and 48th hours the rabbit dies from acute pneumococcal septicæmia.

It is probable (Wright 1927) that the initial rapid clearing which follows the intra-

venous injection of a large dose of virulent pneumococci is the result of the lag phase in bacterial growth (see Chapter 4). Wright has shown that this initial clearance of virulent pneumococci can be almost eliminated by injecting a culture that is still in the logarithmic phase.

In the argument from analogy, which plays so large a part in the construction of our concepts of infection and resistance, we must be very careful to allow for the effects of our experimental technique. The reactions which we have described above are those that follow the sudden introduction into the blood stream of a relatively enormous number of bacteria—a number of the order of 1,000,000,000. Such an occurrence must be of extreme rarity in natural infection, and it is possible that some part of the mechanism we have described—for instance, the aggregation of bacterial cells and the retention of the aggregates in the lung capillaries—may have been over-emphasized by the particular experimental procedure that we have chosen to employ. In any case there seems little doubt that the most significant happenings are those that occur between the 5th and 24th hours after an intravenous inoculation, when the fate of the host is being determined by the balance between bacterial multiplication, on the one hand, and the removal of the newly produced bacterial cells on the other.

Even when the tide has for the moment turned decisively in favour of the host, it does not follow that there will be a complete sterilization of the tissues. Bacteria will have been caught up in the liver, spleen and elsewhere, and there some of them may remain alive, but relatively inactive, for considerable periods.

Our knowledge of the exact method by which bacteria are *killed* in the tissues is woefully incomplete. There is no doubt at all that the majority of the bacteria taken up by phagocytic cells are destroyed by intracellular digestion ; but the tacit assumption that a bacterium phagocytosed is, of necessity, a bacterium finally disposed of is certainly unwarranted.

The Reactions that follow the Intraperitoneal Injection of Bacteria or of Suspensions of Inert Particles into Normal Animals.

The small quantity of fluid present in the normal peritoneal cavity, or other serous sac, contains very few cells, and these consist almost entirely of mononuclears, mainly of the lymphocyte type (Dudgeon and Ross 1906, Cappell 1930, and many others).

Durham (1897), in his classical paper on the reaction to intraperitoneal infection, described the course of events that follows the injection of bacterial and other suspensions into the peritoneum of the guinea-pig. As regards the changes in the cellular contents of the exudate, there is a preliminary period lasting for about 1 hour or less during which no increase in cells can be detected. The cellular reaction, when it occurs, differs widely in degree and in character according to the kind of inoculum employed. With injections of dead bacteria, or of living strains of low virulence, there is a rapid emigration into the peritoneal cavity of polymorphonuclear leucocytes (or *microphages*, if we employ the terminology of Metchnikoff) which ingest the bacterial cells. These cells increase in number up to the 8th hour or later, and then slowly decline. As they decline, their place is taken by large mononuclear phagocytes (*macrophages*), which ingest those bacteria that still remain free in the fluid, together with many of the polymorphonuclear cells and the bacteria that they have already phagocytosed. Durham puts the duration of the whole period of peritoneal reaction, from the time of the first appearance

of the microphages to the final disappearance of the macrophages and the return to normal, as 4 to 7 days.

Although this microphage-macrophage succession may be regarded as the modal reaction in the peritoneal exudate following the injection of a bacterial suspension, it requires considerable modification in the light of subsequent studies (Dudgeon and Ross 1906, Buxton and Torrey 1906*a*, *b*, *c*, *d*, Cappell 1930). The cellular content of the exudate during the earlier hours of the reaction is by no means confined to polymorphonuclear cells, and other cells present during this period may be actively phagocytic. Cappell (1930) believes that the appearance of typical macrophages during the later stages of the reaction is due not to the emigration of fully formed cells of this type from some neighbouring depôt of histiocytes, such as the omentum, but to the gradual maturation of the small mononuclears that are present in the exudate from the earliest stages. These he regards as immature macrophages, and he believes them to be derived mainly from the small mononuclear cells that are so plentiful in the *tâches laiteuses* and adventitial sheaths of the omentum and mesenteries (see also p. 1044).

These events in the fluid exudate form only a part, perhaps only a minor part, of the general peritoneal reaction. Certain specialized regions of the peritoneal membrane, and of the tissues which are subjacent to it, play a very important rôle. Among these specialized tissues the omentum is pre-eminent (Durham 1897, Buxton and Torrey 1906*d*, Dudgeon and Ross 1906). Just as particulate material injected into the circulation tends to be aggregated into masses that are caught up in the lung capillaries, so the particles or cells in a suspension injected into the peritoneum are aggregated into masses that are deposited on the omental surface; and, just as the polymorphonuclear cells of the circulating blood tend to collect in the lung capillaries together with the aggregated particles, so such cells as are present in the peritoneal exudate tend to collect on the surface of the omentum. On this surface, and in the interstices of the underlying tissue, macrophages are normally present, especially in the milk-spots, or *tâches laiteuses*; and these cells play from the first an active part in the phagocytosis of the deposited particles or cells. Ingestion completed, these cells migrate into the deeper omental tissues, changing in shape as they do so from the spheroidal or slightly irregular form of the macrophage of the body fluids or surfaces to enormously elongated trailing cells, which make their way through the interstices of the tissues. When such cells have phagocytosed large amounts of some indigestible material, such as carbon particles, they may be detected in the tissues for periods of weeks or months. The part played by polymorphonuclear cells in the omental reaction appears to vary with different inocula. According to the observations of Buxton and Torrey, they are absent or very scanty during the first few hours or so, but they then begin to collect in increasing numbers and to play their part in the phagocytic reaction.

These reactions at the surface of the omentum, and in its interstices, are rendered doubly effective as a clearing mechanism by the movements of the omentum as a whole following the injection of a suspension of particles or bacteria into the peritoneal cavity. Disposed at first as a more or less extended fold, hanging down among the intestinal coils, it is gradually gathered up into a closely folded band, extending across the peritoneum, and carrying with it, as in a retracted net, the particles caught on its surface layers. The mechanism of these movements has been studied by Florey and Carleton (1926). They find that the omentum is totally incapable of any intrinsic movement. The movements that it undergoes are impressed on it by (*a*) posture, (*b*) the peristaltic movements of the intestines, and (*c*) the movements of the diaphragm. The retraction into a tightly gathered transverse band is due to the alteration in the character of the omental surface, and parti-

cularly to the deposition of fibrin, which causes the omental folds that are brought into apposition to adhere to one another, instead of extending again in response to the next passive movement.

The reaction that follows an intraperitoneal injection is not, however, limited to the peritoneal cavity. Indeed, it spreads beyond it with surprising rapidity.

Muscatello (1895) injected carmine suspensions into the peritoneum, killed the inoculated animals after various intervals, and studied the distribution of the dye. Within 1–2 hours he found particles of carmine in the liver and spleen. Similar particles, in smaller numbers, were found in the lungs, pancreas and testis. Durham (1897) noted the rapid passage of bacteria from the peritoneal cavity to the blood stream, and concluded that the most important route of transit was *via* the diaphragmatic lymphatics, the anterior mediastinal glands and the right lymphatic duct. The importance of the diaphragmatic route was also noted by Dudgeon and Ross, and the correctness of Durham's conclusions has been confirmed by many subsequent observers, notably in the careful studies of Buxton and his co-workers, and in the more recent studies of Bolton (1921). Buxton and Torrey (1906*b*) found that, almost immediately after the intraperitoneal injection of an Indian-ink suspension, there was a rush of carbon particles into and through the lymphatics of the diaphragm. Thence they passed rapidly through the anterior mediastinal lymphatics and the corresponding lymph glands, reaching the blood stream within a very few minutes. During the earliest stages of this transit there was very little evidence of phagocytosis in the mediastinal glands or elsewhere; but, as time passed, an increasing number of carbon particles were found within the histiocytes of the lymph nodes.

In other experiments (Buxton 1906, Buxton and Torrey 1906*a*) the fate of living typhoid bacilli, injected intraperitoneally into rabbits, was followed by quantitative plating methods. The living bacilli passed into the general circulation almost immediately; and their numbers per ml. of circulating blood reached a maximum after about an hour. After this there was a rapid decrease, and few bacilli could be recovered from the blood after about the 6th hour. When suspensions of liver and spleen tissue were plated it was found that there was a rapid accumulation of bacilli in these organs, and particularly in the liver, within a few minutes after an intraperitoneal injection. There was then a transitory decrease in the numbers of viable organisms, followed by a secondary increase lasting from the 2nd to the 6th hour, *i.e.*, the bacilli were increasing in the liver and the spleen while they were decreasing in the blood. After about the 6th hour there was a general decrease, lasting for several days, and more rapid in the liver than in the spleen. When animals were killed 4 days after injection viable bacilli were still recovered from the liver, though in small numbers; larger numbers were recovered from the spleen, and very large numbers from the mediastinal glands. Many observations on laboratory animals have shown that living bacilli may be recovered from the spleen weeks or months after experimental infection (Topley and Wilson 1923, Price-Jones 1927).

It may be noted that the rapid transit of particles or bacteria from the peritoneum to the blood stream is almost certainly the result of the mechanical pumping action of the rise and fall of the diaphragm in breathing. This is probably assisted by a special arrangement of the endothelial cells of the peritoneal membrane covering the diaphragm, which provides for a rapid passage of particles to the underlying lymphatics (Florey 1927).

The Reactions that follow the Injection of Bacteria or Inert Particles into the tissues of a Normal Animal.

By injecting bacteria into the blood stream or the peritoneal cavity, we are in a sense by-passing a stage that must occur in the majority of natural infections. After penetrating the epithelial layer, whether it be of skin, respiratory, alimentary

or genital tract, the particulate infecting agent comes to lie in the intercellular spaces of subepithelial tissues, or, as we saw in Chapter 44, it may rapidly penetrate into a particular type of cell. The usual sequence of events, the induction of an inflammatory process by the toxins of the bacterium, the mobilization of microphages and macrophages, exudation and in some cases the formation of fibrin clots, may result in a localization of the invading organisms, and their gradual destruction by the antibacterial action of certain fluids and cells of the host. Sometimes the local defences are overcome, and the bacteria are disseminated *via* the lymphatic capillaries to the lymph nodes, and, if they are not held there, from thence to the blood stream, with the establishment of a generalized and perhaps fatal disease. But though localization of the disease process may clearly be a reaction tending to promote the survival of the animal infected, localization of the primary infecting dose is not necessarily so. It is arguable that immediate dissemination of the infecting bacteria, so that they are " diluted " in the healthy antibacterial tissues of the host, may ensure their destruction more certainly than their localization in a site where perhaps they may multiply and ripen into a focus of infective particles that will later be disseminated with fatal results. The number of bacteria that get into the tissues at the start of a natural infection is likely in most cases to be small, and it is to be noted that when small inocula are injected experimentally, a given dose of bacteria may be less noxious when " diluted " directly in the blood stream, than as an injection into the tissues.

Lange and Gutdeutsch (1928) found that pneumococci, streptococci and pasteurellæ were more fatal to mice when injected intraperitoneally than when injected subcutaneously or intravenously, and that the subcutaneous was rather more fatal than the intravenous route. In the case of *Erysipelothrix rhusiopathiæ*, the bacillus causing swine erysipelas and mouse septicæmia, the subcutaneous route of injection was found to be more fatal than the intraperitoneal or intravenous. According to Sobernheim and Murata (1924), the dose of *B. anthracis* required to kill a guinea-pig after intravenous or intraperitoneal inoculation is about ten times the dose that will produce a fatal infection when injected by the subcutaneous or intracutaneous routes. Whether or not it is beneficial that particles should reach the blood stream as soon as possible, or be held locally, we must examine the factors likely to promote both events. At the outset we may note that the passage of particles from the tissues directly through the capillary endothelium appears to be unlikely, except in rare cases of gross mechanical trauma to the capillaries, and perhaps in infections with organisms that proliferate selectively in the endothelium itself. As a rule both particles and bacteria reach the circulation by a less immediate route.

The Permeability of Blood and Lymph Capillaries to Foreign Matter.—Underlying the various epithelial surfaces of the body there is an extensive anastomotic network of blood capillaries and lymphatic channels. The two networks, with rare exceptions, constitute independent systems, each lined by a continuous sheet of endothelial cells. The lymphatic network drains into the afferent lymphatic channels, which in turn lead to a lymph node (Drinker and Yoffey 1941). In normal tissues, excepting the liver, the permeability of both capillary and lymphatic endothelium is poor. Substances of low molecular weight only are absorbed from the tissue by the capillaries. For instance, Rous and his colleagues (Rous, Gilding and Smith 1930, Smith and Rous 1931*a*, *b*, Rous and Smith 1931) showed that certain dyes passed directly into the venous portion of a capillary

loop. Cobra venom, of molecular weight 5,000, is absorbed directly into the blood stream, but diphtheria and tetanus toxins, of molecular weights greater than 20,000, are not (Barnes and Trueta 1941). Serum proteins do not pass directly into the blood stream from the tissues (Starling 1895–96). The permeability of the blood capillaries increases greatly with trauma, and under the influence of various noxious substances. It does not appear that the passage into the blood stream of particles of bacterial dimensions is facilitated in these circumstances; though Tuttle and Cannon (1935) obtained some evidence that virulent streptococci passed directly into the blood stream after their introduction into the lung alveoli of dogs. The lymphatics, on the other hand, readily become permeable to large molecules and small particles introduced directly into the intercellular tissue spaces, whether they are, for example, inert particles, red blood cells or bacterial in nature (see, for instance, Cappell 1929, Batchelder, Field and Drinker 1931, McMaster and Hudack 1934, Field and Drinker 1936, Schulz, Warren and Drinker 1938, Barnes and Trueta 1941). We have already noted the speed with which bacteria reach the thoracic duct *via* the lymphatics, from the peritoneal cavity, etc. These facts suggest that in areas which, like the skin and subepithelial tissues of the respiratory and alimentary tracts, are liberally supplied with lymphatics, invading bacteria in many cases are rapidly carried to the lymph nodes draining the site of invasion.

The Filtering Action of Lymph Nodes.—As Drinker, Field and Ward (1934) showed, hæmolytic streptococci or particles of india ink introduced into an afferent lymph channel are to a large extent removed from the lymph during its passage through a lymph node. The filtration appears to depend mainly on the complex ramifications of the channels in the node, and to a lesser extent on the removal of particles by phagocytic cells lining the sinusoids of the node. The filtration, however, is by no means complete. Schulz, Warren and Drinker (1938) instilled virulent Type III pneumococci into the nose of rabbits, and recovered cocci from the vessels draining the superior deep cervical node within one hour of the instillation; non-virulent organisms, on the other hand, did not appear in the efferent lymph. Where lymph has to traverse several nodes in its passage to the thoracic duct filtration may be very efficient. Thus Drinker, Field and Ward (1934) passed large numbers of streptococci through the popliteal and iliac lymph nodes of a dog; 14 ml. of a culture containing at least 250×10^6 organisms per ml. were perfused in 88 minutes; at the end of 56 minutes, 1 ml. quantities of thoracic lymph were sterile, and even after 88 minutes' perfusion, the number of streptococci per ml. was only of the order of 100.

It seems probable that the nature of the particle affects the efficiency of filtration. Thus, Yoffey and Sullivan (1939) found that vaccinia virus instilled into the nose of rabbits appeared in the thoracic duct after a delay of twelve hours, and continued to do so for seven days after instillation. It is not known whether this represented a ready passage of virus through the node, or its retention and multiplication in the tissues of the node, with subsequent discharge into the efferent lymphatics.

As we have seen, the introduction of infective particles into the tissues is usually followed by varying degrees of local inflammatory reaction, and it is pertinent to inquire how far these affect the fate of the particles. Our knowledge of the value of the inflammatory reaction as a defence against infection is still very incomplete. In recent years, however, interest in this aspect of inflammation has been stimulated by extensive analyses of the inflammatory processes made by Menkin in the United States. Briefly, Menkin's view of the process of acute inflammation is as follows.

At the site in which inflammation develops, three substances are liberated from the tissues: leukotaxine, a crystalline polypeptide that increases the permeability of the blood capillaries and is positively chemiotactic, thus attracting polymorphonuclear cells to the inflamed area (Menkin 1936, 1938); a pseudoglobulin which acts on the bone marrow and induces a polymorphonuclear leucocytosis (Menkin 1940*a*, *b*, Menkin and Kadish 1942); and a toxic euglobulin called "necrosin" (see Chapter 44) which is responsible for the necrosis of tissue in the inflammatory area (Menkin 1943*a*). According to Menkin, predominance of any one type of phagocyte at a given stage of the inflammatory process is a function of the metabolic state of the inflamed region. In the early stages, the pH is 7·0 or more, and polymorphonuclear cells predominate; in later stages, owing to the breakdown of carbohydrate in the area, a lactic acidosis occurs, and the resultant fall in pH leads to the appearance of macrophages (Menkin and Warner 1937, Menkin 1943*b*).

Apart from the necrotic effect of necrosin, each of these phenomena can be said to have survival value for an animal attacked locally by an infective parasite. Through the capillary walls made permeable by leukotoxine come phagocytes, and an exudate containing antibodies, other antibacterial substances and fibrinogen. The coagulation of the fibrinogen in this exudate leads to the deposition of a fibrin meshwork in the intercellular spaces of the injured tissues. The protein content of lymph will rise, since under the influence of leukotaxine the lymphatic endothelium will have become permeable to the exuded proteins, and under the toxic action of the invading bacteria the lymph will clot in the lymphatic channels. In favourable circumstances, therefore, the intercellular dissemination of the infective material will be hindered by the fibrin deposit in the tissues, and dissemination along the lymph channels by the intralymphatic clots. This clotting process constitutes Menkin's "lymphatic blockade" whereby irritant material is fixed and held at the site of inflammation. If the irritant is sufficiently strong, "fixation" may even precede the diapedesis of leucocytes. According to Menkin, bacteria like *Staph. aureus* stimulate an early formation of a lymphatic blockade, and hence tend to be confined in local abscesses; *Str. pyogenes*, on the other hand, induces a late fixation reaction, and is conspicuously invasive.

The presence of leukotaxine-like polypeptides in the breakdown products of tissue proteins has been demonstrated by Duthie and Chain (1939), and many of the experimental phenomena upon which Menkin bases his hypothesis of inflammatory fixation have been reproduced by other workers, though neither Steinberg and Dietz (1938) nor Lurie (1939) could find any constant association between pH and the predominating type of phagocyte in inflammatory processes. It is probable that a number of factors, including the type of parasite, determine the cellular response, even in the early stages of infective inflammation (see, for example, Gins, Kroemer and Link 1938).

We are chiefly concerned here with the hypothesis of the lymphatic blockade as a defence against bacterial invasion. The chief objection to the hypothesis is the difficulty of demonstrating fibrin deposits and lymphatic thrombi at the *periphery* of inflamed regions, that is, in the region where they are likely to be effective as a barrier against the spread of infection. Rich (1936) points out that acute inflammatory processes induce an increased, not a decreased, flow of lymph from the injured part (see Field, Drinker and White 1932, Hudack and McMaster 1933), and suggests that foreign substances, including bacteria, are "fixed" in inflamed areas by adsorption to proteins, and by precipitation or other physico-chemical change in the lesion (see also Blalock and Burwell 1935–36); and the work of Miller (1938) on the adsorption of a variety of substances, including bacterial suspensions, from regions of inflammation in rabbits,

makes it clear that absorption depends largely on the nature of the substance absorbed. Miles and Miles (1943) attribute the apparent fixation of substances in the inflammatory lesion to an accumulation of exudate in the tissues to such a degree that the existing lymphatic system can drain away only a small fraction during the period of experimental observation, with the result that any foreign material diluted in the exudate appears also to pass slowly from the lesion.

We should at this point emphasize that the term "fixation" is also used for two phenomena that are distinct from the fixation discussed above. The first concerns the localization of a blood-borne infective agent in the tissues, with the formation of a local lesion. The mechanism of this fixation, as the review of Burrows (1932) shows, is the escape of the infective material through capillary endothelium, the site of escape in most cases being determined by injury to the vessel wall. The fate of the material from this point will be determined by the factors we have already discussed. Another kind of fixation occurs in the later stages of a local infection, in the sense that the whole infected region is walled off from the rest of the body by the cellular reaction. In this case we are dealing with localization of an established *lesion*, a process which is conveniently distinguished from that we have discussed above, which is the localization of infective material soon after its introduction.

It should be noted that lymphatic circulation is largely dependent on active movement of the animal body (see Drinker and Yoffey 1941), and in the quiescent animal, on the movements transmitted through the tissues by the pulsating blood vessels (Parsons and McMaster 1938). It follows, as Barnes and Trueta (1941) have demonstrated, that the complete immobilization of a limb in plaster will greatly retard the adsorption by the lymphatics of large particles, and of substances of high molecular weight, injected into the limb.

To summarize, it is frankly impossible at present to forecast the fate of infective particles introduced into the tissues—whether they will be immobilized or carried *via* the lymphatic system to the blood. Nor can we predict which of the two results would be more effective in destroying the infective agent. Clearly both local and systemic defence mechanisms are highly effective in certain circumstances. In our opinion the known facts are inadequate for a generalization like that of Menkin on the rôle of inflammation in immunity, though the phenomena he has described are manifestly of first importance.

The Reactions that follow the Administration of Bacteria or Inert Pathogenic Particles to Normal Animals by the Respiratory Tract and by Mouth.

Our studies of the reactions following the injection of material into the tissues indicate that in most cases particles over a certain size gain access to the lymphatics, and if a lymph flow is established, rapidly reach the lymph nodes. The extensive lymphatic system of the skin is protected by a relatively thick and impermeable dermis; the equally extensive submucous lymphatic systems of the respiratory and alimentary tracts are covered by a much more penetrable mucosa.

Particles as large as globulin molecules readily penetrate the nasal mucosa and rapidly reach the lymphatics (Drinker and Yoffey 1941). Few observations have been made on the fate of viruses and bacteria in the nose. As we have seen (Yoffey and Sullivan 1939), vaccinia virus did not appear in the lymphatics until 12 hours after its introduction into the rabbit's nose, suggesting that multiplication in or on the mucosa preceded its penetration. Yoffey and Drinker (1939) could find no evidence of the lymphatic penetration by the poliomyelitis virus (see Chapter 87). Schulz, Warren and Drinker (1938)

demonstrated a ready penetration of the lymphatics, both of the nose and the trachea, by virulent pneumococci.

We shall discuss the reactions of the alveolar tissues of the lung to bacterial invasion in Chapter 74, in connection with the pathogenesis of pneumococcal pneumonia. With regard to infections by the mouth Ørskov and his colleagues (Ørskov, Jensen and Kobayashi 1928, Ørskov and Moltke 1928, Ørskov and Lassen 1930), extending the observations of Müller (1912), have recorded a series of experiments in which they fed mice with *Salm. typhi-murium* and allied strains of the *Salmonella* group by dropping measured amounts of a broth culture, or of a saline suspension, into the open mouth. The animals so treated were killed after intervals varying from a few hours to a month or more, and cultures were prepared from the blood, liver, spleen, mesenteric glands, the small intestine at different levels, and certain other situations. In this way it was possible to follow the spread of infection throughout the body. These observations are of particular interest because they are concerned with a natural disease of mice, and because the portal of entry is that by which the parasite gains access to its host in the natural spread of the disease.

It would seem that the bacilli, when they gain access to the body by the mouth, fail to establish any immediate foothold in the intestine, and for the most part rapidly succumb. A certain number, however, enter the tissues from the alimentary tract and are carried to the mesenteric lymph glands. Later they enter the blood stream, probably *via* the thoracic duct, but are rapidly removed from the circulation by the reticulo-endothelial cells, particularly those of the liver and the spleen. During this phase the blood taken from the heart is sterile, and *Salm. typhi-murium* cannot usually be recovered from the intestine; while the liver, spleen and mesenteric glands show its presence in increasing numbers. In many animals this stage is followed by a secondary bacteræmia, which increases in intensity until the animal succumbs. The intestine, which as stated above is rapidly freed from the invading bacteria during the primary stage of the infection, becomes secondarily infected during its later stages, probably by way of the bile-duct.

A similar series of experiments were carried out using *Salm. paratyphi B* as the infecting agent—an organism closely related to *Salm. typhi-murium*, but far less pathogenic for the mouse. The same rapid disappearance from the intestine was noted, and the same localization in the lymphatic glands; but in this case there appeared to be no tendency for the infection to spread beyond this primary focus, though the bacilli might persist in the mesenteric glands for weeks or months. A relatively avirulent variant of *Salm. typhi-murium* behaved in the same way as *Salm. paratyphi B*.

Bactericidal and Bacteriolytic Reactions.

It is very difficult to assess with any accuracy the part played by the lytic action of complement on sensitized bacterial cells. There is little doubt that this purely humoral effect is much less important than was at one time supposed, but it is certainly operative to some degree with certain organisms and under certain conditions.

Thus, apart from Pfeiffer's classical experiments with *V. choleræ*, Buxton and Torrey (1906*d*) note that many of the typhoid bacilli that collect on the surface of the omentum after an intraperitoneal inoculation are destroyed by extracellular lysis instead of being ingested by macrophages, and many workers have recorded analogous observations. In the case of Gram-positive organisms, such as pneumococci or streptococci, it would seem, however, that this purely humoral mechanism plays no part.

THE REACTION OF ACTIVELY IMMUNIZED ANIMALS AS COMPARED WITH THAT OF NORMAL ANIMALS

We have so far been concerned only with the reaction of normal animals to bacteria of varying virulence ; and we have seen that the normal clearing mechanism, while highly effective against a strain that has little capacity for multiplication within the tissues, may be overwhelmed by a more virulent strain that is capable of establishing multiple or extensive foci of active infection from which the circulation is continuously flooded.

So far as this particular mechanism is concerned, we can summarize the difference between an actively immunized and a normal animal very briefly, by saying that an immunized animal behaves towards a virulent strain of a particular pathogenic bacterium in the same way as a normal animal behaves towards an avirulent, or

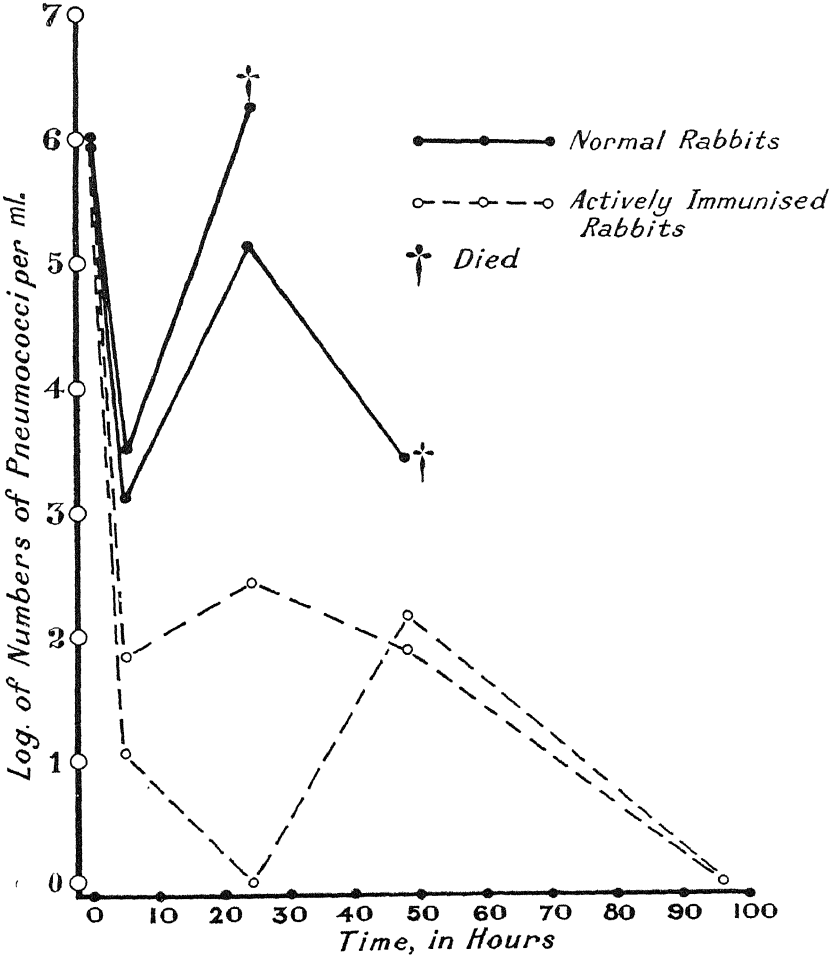


FIG. 241.

slightly virulent, strain of the same bacterial species. Without any attempt to recapitulate in detail, we may quote a few illustrative examples.

Wright (1927) studied the response, to the intravenous injection of virulent pneumococci, of rabbits that had been immunized by the injection, at various intervals before the test inoculation, of a killed culture of the same strain. Table 70 and Fig. 241 show the results obtained in two rabbits that had been immunized 3 months previously, and in two normal controls injected with the same dose of the same living culture. Comparison with Table 69 and Fig. 240 will show that the immunized rabbits dealt with the highly virulent culture in the same way as the normal rabbit dealt with the slightly virulent strain.

TABLE 70

SHOWING THE NUMBER OF PNEUMOCOCCI PER ML. OF CIRCULATING BLOOD AT VARIOUS TIMES AFTER INOCULATION OF A VIRULENT STRAIN INTO NORMAL AND INTO ACTIVELY IMMUNIZED RABBITS.

Time after Injection.	Normal.		Immunized.	
	Rabbit 247.	Rabbit 248.	Rabbit 299	Rabbit 300
Immediately	870,000	1,100,000	1,000,000	1,000,000
5 hours	1,300	3,300	12	68
24 ,,	142,000	1,953,000	0	289
48 ,,	2,800	Innumerable	149	79
96 ,,	Dead	Dead	0	0

The phagocytic reactions that we have described are illustrated in Figs. 242, 243, 244, and 245, for which we are indebted to the late Professor H. D. Wright.

The reaction in the lung capillaries in these immunized animals is illustrated by the following figures (Wright 1927) obtained from a film preparation.

Total number of pneumococci seen	893
Phagocytosed by polymorphonuclear cells	297
Phagocytosed by mononuclear cells	340
Total phagocytosed	637
Total outside phagocytes	256
In unphagocytosed aggregates associated with platelets	206
In free aggregates	53

The results that have been obtained when immunized animals have been injected with virulent bacteria by other routes—by intraperitoneal or subcutaneous injection or by the mouth—are in entire conformity with those described above. An immunized animal reacts to a highly virulent bacterium as does a normal animal to one of lower virulence. The exact degree of difference in behaviour depends on the grade of immunity that has been established.

We may cite as further illustrative examples of the reactions of normal and immune animals the studies of Angevine (1936) on intradermal infections by virulent streptococci and the detailed studies of Jawetz and Meyer (1944*a*, *b*) on the reactions of the guinea-pig to infection by plague bacilli.

The immunity with which we are here concerned is, it should be noted, strictly specific. The influence of non-specific factors on immunity is considered in Chapter 52.

The Passive Transference of Antibacterial Immunity.—The increased resistance that an actively immunized animal enjoys in virtue of the increased efficiency of the defence mechanism considered above can be passively transferred from an immunized to a normal animal by injecting into the second the blood serum of the first.



FIG. 242.

Showing phagocytosis of pneumococci by macrophages in the liver of an immunized rabbit.



FIG. 245.

Lung of an immunized rabbit, showing phagocytosis of pneumococci in capillaries of alveolar walls.



FIG. 243.

Showing phagocytosis of pneumococci by the Kupffer cells, from the liver of an immunized rabbit.



FIG. 244.

Blood from the liver of an immunized rabbit, showing phagocytosis of pneumococci.

It follows that we must regard the mechanism by which virulent bacteria are disposed of when once they have gained access to the tissues of an immunized animal as involving an integrated system of cellular reactions conditioned by the presence of specific antibodies in the blood or tissue fluids.

Again, a few illustrative examples will suffice.

Bull (1915*a*, *b*) found that, in a rabbit in which a bacteræmia had been established by the intravenous injection of pneumococci, the bacteria were removed from the circulation within about 15 minutes after the intravenous injection of 0·2–0·5 ml. of antipneumococcus serum per kilo-body-weight of the rabbit.

Wright (1927) showed that the clearing mechanism of a normal rabbit could be rendered highly effective against virulent pneumococci by the intravenous injection of the serum of an actively immunized animal. The results of one such experiment are set out in Table 71 and Fig. 246. The two passively immunized rabbits received 1 ml. respectively of the serum of an actively immunized rabbit 1 hour before the test dose of culture. It will be noted that the normal animal died between the 5th and 24th hours, so that the expected secondary rise in the number of bacteria was not observed.

TABLE 71

SHOWING THE NUMBERS OF PNEUMOCOCCI PER ML. OF CIRCULATING BLOOD AT VARIOUS TIMES AFTER INOCULATION OF A VIRULENT STRAIN INTO ONE NORMAL AND TWO PASSIVELY IMMUNIZED RABBITS.

Time after Injection.	Normal.	Pass. Imm. 1.	Pass. Imm. 2
Immediately	2,300,000	2,300,000	2,000,000
5 hours	43,000	2	52
24 „	Dead	8	14
48 „	—	0	1
96 „	—	0	0

Manwaring and Coe (1914) perfused a normal liver with a dilute suspension of pneumococci, with and without the addition of an antipneumococcal serum, and found that the retention of pneumococci within the liver capillaries was very marked in the former case, but very slight in the latter.

There are many earlier observations of an analogous kind.

Bordet (1897) showed that an antistreptococcal serum would protect a guinea-pig against the intraperitoneal injection of a lethal dose of living streptococci. By withdrawing samples of the peritoneal exudate at various intervals after the inoculation, he was able to show that the recovery of the passively immunized animal was associated with a greatly increased degree of phagocytosis, mainly by the polymorphonuclear cells. By a very ingenious experiment he demonstrated that the relatively slight degree of phagocytosis in the peritoneal exudate of the normal animal could not be ascribed to an absence of activity on the part of the leucocytes. If a suspension of *Proteus* bacilli were injected into the peritoneum of a normal guinea-pig at a time when virulent streptococci were multiplying actively therein without undergoing any considerable degree of phagocytosis, the leucocytes at once ingested the *Proteus* bacilli in enormous numbers.

Pfeiffer and his colleagues (Pfeiffer 1893*a*, *b*, 1894*a*, *b*, 1895, Pfeiffer and Issaeff 1894, Issaeff 1894) showed that an anticholera serum would protect a guinea-pig against the intraperitoneal injection of living cholera vibrios; but in this instance the protective effect would appear to depend mainly on a lysis of the vibrios, brought about by the combined action of antibody and complement.

It has, then, been shown quite clearly that specific antibodies play an important rôle in antibacterial immunity. Are they always concerned, or is there some other defence mechanism of the tissues that functions independently of them ?

The evidence at present available does not allow us to give any decisive answer to this question ; and reference to the literature shows a wide divergence between the tentative conclusions sponsored by different observers. One reason for this is

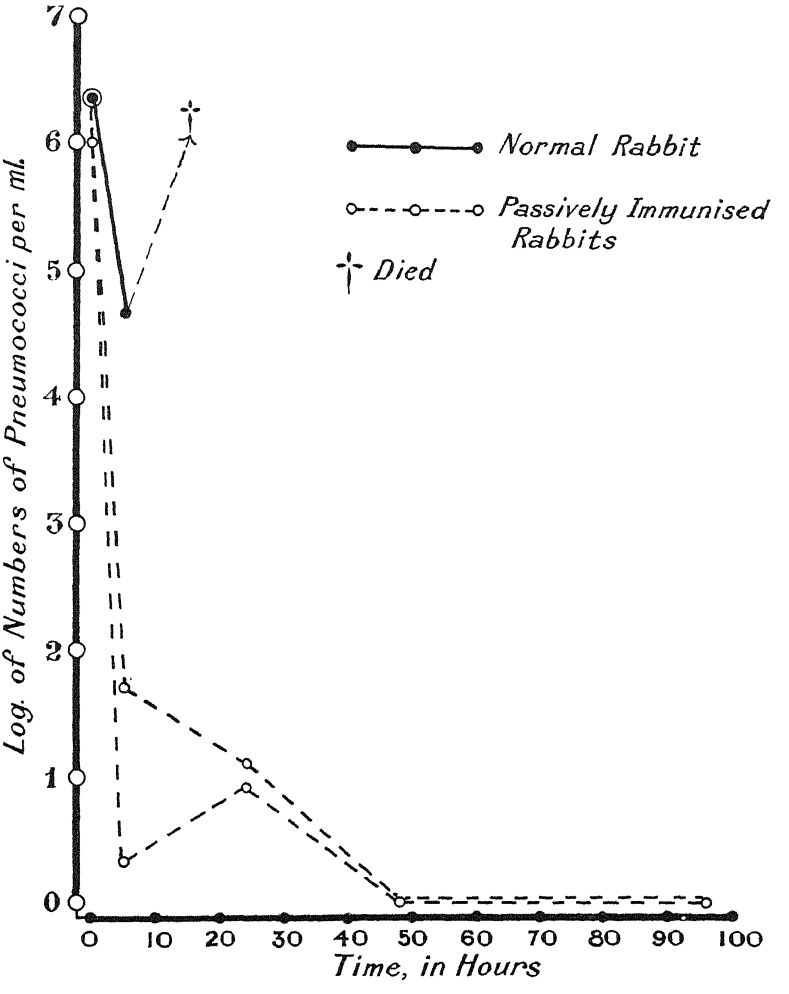


FIG. 246.

that the point at issue has not always been clearly defined. Sometimes the problem actually submitted to experimental study has been the correlation between resistance to a particular bacterium and the presence of the corresponding antibodies in the circulating blood. But we shall see, when studying anaphylaxis, that specific antibodies readily become fixed to tissue cells ; so that the demonstration of immunity in the absence of circulating antibodies, or a failure to find any correlation

between antibody titre and degree of resistance, cannot be accepted as satisfactory evidence that specific antibodies are not involved.

As an illustrative example of the relations that have been found to hold in experiments of this kind we may refer again to the careful studies of Wright (1927).

In these experiments, a large series of rabbits were immunized by a single intravenous inoculation of killed culture and were tested at various intervals thereafter by determining the rate at which virulent pneumococci disappeared from the blood stream, after the intravenous injection of 1 ml. of living culture. The results suggested that there was a slight, but definite, improvement in the clearing capacity within a few hours. This became well established on the 3rd day, was still marked at the end of a month, and might persist for 3 to 6 months, though in diminishing degree. The serum of many of these rabbits was tested for the presence of agglutinins, opsonins, and precipitins. It was found that the production of these antibodies was inconstant, and that the times of their appearance and disappearance in the blood in no way corresponded to the duration of the improved clearing capacity. In many cases no antibodies were ever demonstrable, although the rabbit showed a well-marked clearing reaction. In others, agglutinins appeared to low titre on the 4th day, increased in amount to the 7th or 10th day, and disappeared by the end of the 3rd week. Thus they first appeared after the increased clearing capacity had become well established, and vanished while it was still present in a marked degree. In connection with these experiments Wright also determined the inhibiting action of whole blood (heparinized) on the growth of pneumococci, using a modification of the methods employed by earlier workers in determining the bactericidal action of whole blood on this organism. He found that the results of this *in vitro* test were far more closely correlated with the clearing capacity of the rabbits, and with the power of their serum to confer this clearing capacity on normal rabbits, than were the results obtained by agglutination or precipitin reactions.

A protocol, taken from his paper, and slightly modified in form, affords a clear illustration of the general type of his results. (Table 72.)

TABLE 72

Rabbits.	Immune A.	Immune B.	Immune C.	Immune D.	Immune E	Normal.	Normal.
Action of Serum.							
Agglutinins . .	—	—	—	—	—		
Precipitins . .	—	+	—	—	+		
Inhibition of growth by whole blood . . .	+	++	++	++	++		
Passive protection of normal rabbits . . .	+	++	+	slight	++		
Clearing capacity of rabbit:	No. of organisms per ml. after intravenous injection.						
At once . . .	1,030,000	1,170,000	1,170,000	1,250,000	840,000	950,000	1,070,000
2 hours . . .	20,800	0	0	0	0	210,000	137,000
5 ,, . . .	340	0	0	0	0	25,000	25,000
24 ,, . . .	1,300	0	0	0	116	1,400,000	1,510,000
48 ,, . . .	134	0	0	0	0	Dead	Dead
96 ,, . . .	0						

The lack of correspondence between the presence of agglutinins and precipitins on the one hand, and clearing capacity on the other, is obvious; but the association between the growth-inhibitory action of the whole blood and the clearing capacity is very close. Similarly, those rabbits which are able to remove pneumococci rapidly and completely from

their own circulating blood are in a position to confer a similar ability on normal rabbits, by the transference to the latter of a relatively small proportion of their blood serum. The correspondence, in this case, is not absolute; but the association is well-marked.

It should, perhaps, be noted that other workers have reported the occurrence of an effective active immunity in the entire absence of circulating antibodies, as judged by all available tests, including the bactericidal power of the whole blood (see, for instance, Teale 1935).

Evidence of an essentially different kind has sometimes been appealed to in the discussion of this problem. It has been pointed out that there may be little if any correspondence between the protective power of an antiserum and its antibody content as determined by agglutination, or other *in vitro* tests (see, for instance, Wadsworth 1917, Avery *et al.* 1917). So far as this is so, it is clearly not an argument in favour of a tissue immunity as against an immunity depending on humoral antibodies—the passive transference of protection is proof enough that some humoral factor is concerned. The most that can be concluded from such observations is that the protective power of the serum is not determined by the particular antibodies measured by the *in vitro* tests. The force of most arguments of this kind has been greatly weakened during recent years by the demonstration that antibacterial immunity is dependent on the antibodies acting on particular antigenic components of the bacterial cell, while other antibodies, though giving agglutination or precipitation *in vitro*, may have no protective effect *in vivo* (see below). It has been shown too that many of the quantitative methods used by the earlier workers were very ill-adapted for the accurate assay of the antibodies concerned; and the employment of a more adequate technique has resulted, in many cases, in the demonstration of a close correspondence between the protective power of a serum and its content in a particular antibody (see Smith 1932).

As an example of an acquired cellular immunity in which the participation of antibodies has been excluded as rigorously as experimental conditions permit, we may cite the recent experiments of Lurie (1942), who injected tubercle bacilli into normal and immune rabbits, and two days later removed specimens of tissue, from which he obtained suspensions of mononuclear phagocytes containing ingested tubercle bacilli. These were injected into the anterior chamber of the eye of normal rabbits, " immune " cells into one eye, " normal " cells into the other. After 10–14 days " normal " cells had proliferated, and the chamber contained large numbers of intracellular and extracellular tubercle bacilli; " immune " cells had not proliferated and only a few degenerate bacilli were seen, mostly intracellular. Viable counts of the contents of the anterior chamber confirmed the inference that the " immune " cells inhibited the growth of tubercle bacilli. It is not, however, clear how far the increased immunity was specific for tubercle bacilli. For example, in earlier studies Lurie (1939) showed that macrophages from tubercle-immune animals were more actively phagocytic *in vitro* than those from normal animals, but that the activity was displayed, not only towards tubercle bacilli, but towards inert particles and other bacteria.

Perhaps the strongest argument in favour of the essential rôle of the serum antibodies in antibacterial immunity is the fact that such immunity is specific. It is true that a non-specific active immunity can sometimes be induced; but it is almost always of the relatively trivial and transient type described in Chapter 52. Effective and durable active immunity is usually narrowly specific; and in many cases we can identify the particular antigenic component, or components, on which it depends. If there is an immunity that has nothing to do with antibody formation, then we must invoke an additional specific mechanism, in which the same antigenic

components are in some way involved. There is nothing very unlikely in such an assumption, and it may well prove to be correct; but it will accord with the sound principle of economy of hypothesis, to reserve judgment pending the accumulation of further evidence.

The Dependence of Effective Antibacterial Immunity upon Particular Antibodies.

On which of the various serum antibodies does antibacterial immunity depend? This question was answered, in respect of one particular infection, long before anyone realized that it had been asked. Neufeld and Händel (1909), in their classical paper on the testing of antipneumococcal sera, first showed the existence of different serological types of pneumococci by noting that a given serum would protect a mouse against one strain of pneumococcus but not against another.

The demonstration of the presence of two separable antigenic components in the pneumococcal cell—the type-specific polysaccharide and the non-specific nucleo-protein—afforded an obvious opportunity of approaching this problem along more satisfying lines; and the results obtained were unequivocal. The antibody corresponding to the polysaccharide hapten was found to have a high protective value: the antibody corresponding to the nucleo-protein antigen had little, if any, protective effect (Avery and Morgan 1925, Avery and Neill 1925). A decisive demonstration of the protective action of the antibody acting on the specific polysaccharide, in the absence of antibodies acting on any other constituent or product of the pneumococcal cell, was more recently recorded by Avery and Goebel (1931). They prepared a synthetic antigen by obtaining purified polysaccharide from a culture of Type III pneumococcus and linking it to horse globulin, and with this antigen they immunized rabbits. The antisera so obtained protected mice against Type III pneumococci, but not against Type I or Type II pneumococci.

In this connection an observation of considerable interest was made by Sugg, Richardson and Neill (1929). It was found that a particular variety of yeast contained the specific polysaccharide component of the Type II pneumococcus. Vaccination of mice with a heated suspension of this yeast significantly increased their resistance to Type II pneumococcus, but not to Type I or Type III.

Goebel (1939, 1940) succeeded in demonstrating an even more precise relationship between specific immunity and the chemical structure of the major pneumococcal antigen. He prepared azo-protein antigens containing the glucoside of cellobiuronic acid, an acid known to be a constituent of Type III and Type VIII pneumococcal polysaccharides, and was able to protect mice against Type III and Type VIII infections by treatment with antisera from rabbits immunized with the azo-protein. In the same way, antibodies to antigens containing the glucosides of synthetic gentiobiuronic and glucuronic acids protected mice against Type II pneumococcal infection.

The predominating rôle of antibody to polysaccharide in type-specific immunity to pneumococcal infections is well established, and it is of interest to note that where the main antigen of a pneumococcal type is complex, the most solid immunity is achieved when antibodies to all the components are present. Kauffmann, Mørch and Schmith (1940) separated the Type VII pneumococcus into four sub-types distinguished by different combinations of eight antigenic components, *a* to *h*. Björneboe (1940) was able to prepare sera specific for some of the separate components, and showed that full protection of mice against Type VII (containing $a + b$) and Type VIIA (containing $a + b + c$) was obtained only when the animals received mixtures containing the full quota of corresponding antibodies, *i.e.*, anti-a + anti-b, and anti-a + anti-b + anti-c, respectively.

Certain workers, notably Day (1933, 1934) and Harley (1935), describe an acid-extractable, species-specific, immunizing antigen in pneumococci. Downie (1938) was unable to find an antigen of this character in pneumococci, though he described an antiprotein antibody with some protective power (see Chapter 54). Street (1942), however, reports that prolonged immunization of rabbits with an R Type II pneumococcus induced a

species-specific antibody that protected the immune animal against intrapleural infections of virulent Type I pneumococci. Its chief effect appeared to be opsonic, particularly in respect of the macrophages.

Julianelle (1926) has reported more or less similar findings in the case of Friedländer's bacillus. A serum containing antibodies for one of the type-specific polysaccharides protects mice against the corresponding serological type. A serum containing only antibodies corresponding to the common nucleo-protein antigen affords no protection. A similar dependence of immunity on type-specific antibodies has been recorded for *Str. pyogenes* (Stamp 1936, Stamp and Hendry 1937) and *Sh. flexneri* (Weil and McFarlane 1944).

It was, however, in the case of the motile bacilli, possessing flagellar and somatic antigens, that the problem of the different immunizing value of different bacterial antigens was first stated in its clearest and most explicit form by Felix and his colleagues (see Felix 1924). The results of subsequent experimental studies have afforded strong support to his contention that the flagellar antigens, and the corresponding antibodies, play little if any part in the mechanism of specific antibacterial immunity, while the heat-stable somatic antigens and the antibodies that react with them are all-important.

Arkwright (1927) immunized guinea-pigs by the injection of vaccines prepared from different strains of *Salm. typhi* and *Salm. paratyphi A*, and subsequently tested their resistance by the injection of living virulent bacilli.

In the case of *Salm. typhi*, a vaccine prepared with a smooth, motile, virulent strain (containing the flagellar antigen and the surface antigen IX) produced a significant increase in resistance. A vaccine prepared from a rough, motile variant (containing the flagellar antigen but not the surface antigen IX) did not. In the case of *Salm. paratyphi A* four vaccines were used: (1) a smooth, motile strain, with the flagellar antigen and the surface antigens I, II, (2) a non-motile, smooth variant, with no flagellar antigen but retaining the surface antigens I, II, (3) a rough, motile variant, with the flagellar antigen but without the surface antigens I, II, and (4) a rough, non-motile variant containing neither the flagellar antigen nor the surface antigens I, II. Vaccines (1) and (2), containing the antigens I, II, produced a marked increase in resistance. Vaccines (3) and (4), from which this antigen was absent, did not.

Ibrahim and Schütze (1928) obtained analogous results in the immunization of mice against *Salm. typhi-murium*. They found that the R variants, in which the specific polysaccharide antigen was absent, were ineffective as immunizing agents. The S variants, containing the specific polysaccharide antigen, produced a significant increase in resistance.

Experiments, in which untreated mice and mice vaccinated with different bacterial suspensions were submitted to the risk of natural infection during a long-continued experimental epidemic of mouse typhoid, have yielded entirely concordant results (Greenwood, Topley and Wilson 1931). The total numbers in each of the vaccinated groups were large —over 300 mice—so that the difference noted may be regarded as certainly significant. The results are summarized in Table 73.

The column giving the antigenic structure shows the components on the surface of the bacterial cells and those carried by the flagella. All vaccines except E were saline suspensions, killed by heat and formalin. Vaccine E—a killed broth culture—was included because of the possibility that some bacterial product of immunizing value might be liberated into the culture fluid. The measure of resistance—the mean survival time in the epidemic cage limited to 60 days—was selected for reasons that will be referred to later. The significance of the standard error, which is attached to each survival time, has been considered in Chapter 43.

TABLE 73

Group.	Vaccine.	Antigens	Mean Survival Time in Epidemic Cage Limited to 60 Days.
C	*Salm. typhi-murium* . . . . . (Smooth—Type and Group)	Surface, IV, V, (XII). Flag. i. Flag. 1, 2, 3.	35·31 ± 0·884
E	*Salm. typhi-murium* . . . . . (Smooth—Type and Group—Broth culture)	Surface, IV, V, (XII). Flag. 1. Flag. 1, 2, 3.	34·97 ± 0·845
F	*Salm. paratyphi B* . . . . . (Smooth—Group)	Surface, IV, V, (XII). Flag. 1, 2.	35·06 ± 0·845
G	*Salm. paratyphi B* . . . . . (Smooth—Type)	Surface, IV, V, (XII). Flag. b.	33·71 ± 0·853
D	*Salm. typhi-murium* . . . . . (Rough—Type and Group)	Surface, R. Flag. i. Flag. 1, 2, 3.	29·41 ± 0·771
B	*Salm. typhi* . . . . . (Rough)	Surface, R. Flag. d.	29·28 ± 0·732
A	*Staph. albus*	—	29·86 ± 0·714
H	Unvaccinated Controls	—	26·26 ± 0·641

It will be seen that the control and vaccinated groups can be divided into three classes on the basis of their survival time. The unvaccinated mice lived, on the average, for 26·26 days, and were thus significantly less resistant than any other group. The groups D, B and A were slightly more resistant. This increased resistance was clearly not specific. The rough polysaccharide antigen was not concerned, for the *Staph. albus* vaccine did not contain it. The flagellar antigens of *Salm. typhi-murium* were not concerned, for neither the *Staph. albus* nor the rough *Salm. typhi* contained them. The groups C, E, F and G were definitely more resistant; their mean survival time varied from 33·71 to 35·31 days. They all differed from the other groups in that they contained the specific polysaccharide surface antigens of *Salm. typhi-murium* (IV and V). They differed among themselves with regard to their flagellar antigens. The results indicate quite clearly that the specific polysaccharide surface antigen is the most important immunizing agent. There is little doubt that the surface antigens of the salmonellæ are identical with the endotoxins that can be extracted from them (see, for example, Raistrick and Topley 1934, Boivin and Mesrobeanu 1936) and, as the experiment set out in Table 73 exemplifies, any cross-immunity induced by different salmonellæ is referable to their having a somatic antigen in common. These antigens stimulate the production of antibodies that have moderate anti-endotoxic powers, and are strongly antibacterial (Boivin and Mesrobeanu 1938*a*, *b*).

In the particular case of the typhoid bacillus it is necessary to discuss the significance of the Vi antigen and Vi antibody, described by Felix and Pitt (1934*a*, *b*) (see Chapter 69). The relation of the Vi and O antigens of *Salm. typhi* to antibacterial immunity is complex. Topley and his colleagues (1937) prepared an antigenic fraction containing a mixture of Vi and O antigens, and one containing the O antigen alone. On injection into mice, both conferred immunity to virulent Vi-containing typhoid bacilli. Later comparisons of relatively pure preparations of Vi and O antigen confirmed their immunological differences. Pure O and pure Vi sera specifically neutralized the *toxic* effect of the corresponding antigens, and exhibited a high degree of cross-protection against *infection* with *Salm. typhi* containing Vi and O antigens (Boivin and Mesrobeanu 1938*c*, 1939, Henderson and Morgan 1938, Boivin 1939). Henderson (1939), however, demonstrated a feebler protective action of O sera in mice when large infecting doses of bacilli were employed. He also showed that against infection with pure Vi and pure O strains of *Salm. typhi* only specific Vi and O sera respectively were protective (see also Grasset and Lewin 1937).

Robertson and Felix (1930) found that an antiserum to *Cl. septicum*, containing antibodies against the heat-stable somatic antigen, but none against the heat-labile flagellar antigens, had a high protective value in mice. Henderson (1932) recorded analogous results with *Cl. chauvœi*. These results accord in general with those found in the salmonellæ. But more recently Henderson (1937) observed that antisera to unheated formolized *Cl. septicum* contained a protective antibody specific for a heat-labile antigen in the bacilli. The antigen had none of the qualities of the heat-labile Vi antigens, and appeared to be flagellar in nature, the protective effect being roughly correlated with the flagellar agglutination titre. Henderson suggests that flagellar antibody might exert an immobilizing effect on a motile, pathogenic bacterium and thus prevent its dissemination through the tissues. If this is the case, it should be possible to demonstrate protection with flagellar antibodies in other types of infection.

The Efficacy of Antibacterial Immunity.

As we emphasized in the earliest pages of this book, immunity may be of very varying grade. The first effect of the mechanisms that we have discussed in this chapter is to free the circulating blood from bacteria, and so save the host from a fatal bacteræmia. The effect on the local foci of infection is less pronounced, and much less rapid. Bacteria may remain latent in such foci over considerable periods.

Thus (Topley 1929) of 64 vaccinated mice that had survived in apparent health for 28 days after an intraperitoneal injection of 1,000 virulent *Salm. typhi-murium*, 31 were found to be harbouring that organism in the spleen. We need not doubt that a complete sterilization of the tissues often occurs; but we must not identify the absence of overt illness with a complete absence of infection, or illness followed by recovery with infection followed by a return to the uninfected state.

There is another factor which may limit the efficacy of immunity of this type. There are regions of the body in which bacterial infections are particularly dangerous, and when foci are established at these sites an immunity that is effective against lesions elsewhere seems to be of little avail. Thus Bull (1915*b*) notes that immunized animals, after rapidly freeing their blood stream of pneumococci and remaining in apparent health for several days, may later succumb to a pneumococcal meningitis. The particular susceptibility of certain regions of the body, even in the immune animal, may be an inherent feature of the animal. It may also be a reflection of variations in the distribution and efficacy of antibody in the various tissues. We shall examine this latter possibility in the next section.

In general, antibacterial immunity is, as we should expect, found to be more variable and often less effective than antitoxic immunity.

The Conditions under which Antibodies exert their Protective Action.

The union of antibody with the surface antigens of an invading bacterium clearly renders the parasite more susceptible to the defence mechanisms of the infected host; for example, to the lethal and bacteriolytic action of complement and to phagocytosis. The importance for protection of the host of phenomena like precipitation and agglutination is less well established. The formation of visible precipitates, or of an agglutinated mass of bacteria, in the tissues or tissue fluids of an immune animal cannot take place unless there is an adequate concentration of antigen, either in the form of soluble products of bacteria, or of bacteria themselves.

In discussing the functional significance of precipitins Cannon (1940) suggests that specific aggregates in the body range in size from molecular aggregates up to large pre-

cipitates. The *in vivo* formation of large precipitates is, however, mostly a matter of conjecture, and while it is not to be doubted that antigen-antibody complexes of a molecular order of size are formed *in vivo*, they are clearly not precipitates. The reaction of the clearing mechanisms of the body to soluble antigen modified by union with antibody probably parallels the reactions to bacteria modified by antibody. As Cannon points out, soluble antigen disappears more rapidly from the blood of an immune animal than from that of a normal animal; but there is little evidence that the aggregates have to reach the size implied in the description "precipitate" before the clearing mechanism can take effect.

It is of interest to note that precipitation apparently plays an important part in immunity to certain parasitic nematodes. Immune sera precipitate with antigens contained in the excretory materials exuding from the mouth and anus of the worms. The resulting interference with the metabolism of the worms is lethal (see Taliaferro 1940, Oliver-González 1940).

Agglutination in the blood stream of the infected animal may be a prominent feature in the experimental tests of clearing mechanisms we have described above. It is, however, observed only with relatively enormous doses of bacteria (see, for example, Reiss 1939). It is probably less prominent in natural infections of the blood stream, where high concentrations of bacteria are unusual. In a local lesion, however, where the concentration of bacteria in the body fluids may be high, it is possible that antibody may help to localize the infection, not only by agglutinating existing bacteria, but by sensitizing growing bacteria so that their daughter cells, when formed, remain in a clump instead of dispersing.

The importance of agglutination as a protective mechanism in experimental pneumococcal infection has been stressed by Oelrichs (1934, 1935, 1936). Rich and McKee (1934) showed that antipneumococcal serum helped to localize experimental skin infections in rabbits whose leucocytic response had been inhibited by benzene poisoning. Wood (1941) found that in rats, the advancing edge of the pneumonic lesion consisted of a leucocyte-free zone of œdema, in which the alveoli contained only fluid and pneumococci. Nevertheless, when antipneumococcal serum was given intravenously, the spread of the infection was largely inhibited, and the pneumococci at the periphery of the lesion were at the same time agglutinated. A phagocytic response appeared later round the agglutinated masses, but prior to this, there was a definite association between localization of the disease, and the appearance in the infected tissue of agglutinating antibody. It is difficult to determine whether in these cases the agglutination is to be regarded merely as a consequence of an antigen-antibody reaction whose chief protective function is sensitization to phagocytosis (when phagocytes are present) and to the bactericidal action of the complement, etc., in the tissue fluids, or whether agglutination by itself is antibacterial. Aside from the purely mechanical effect of aggregation into large masses that are not readily dispersed through the tissues, there is little other protective function we could assign to agglutinins *per se*. Oldfelt (1942) reviews the evidence that bacteria are damaged by the action of agglutinins on living bacteria *in vitro*. Agglutination appears to have little effect either on the viability or on the respiratory activity of bacteria, except when the agglutinated masses are large. In these circumstances, Surányi and Pálóczy (1930), von Jenny and Váczi (1940) and Oldfelt himself recorded a diminution in the rate of oxygen uptake. At most, then, we may postulate that agglutination *in vivo* may be directly antibacterial when the agglutinated masses are so large as to interfere with the metabolism of bacteria in the centre of the masses.

The Availability of Antibody in the Tissues of the Host.

The concentration of antibody in the blood is one of the simplest indications of a state of immunity, and it is possible to determine by experiment the relation

between a given blood concentration and a specifically immune state of the animal. This immunity, however, depends on antibacterial qualities not only of the blood, but also on that of the tissues, the intercellular fluids, lymph, and so forth. Moreover, in most natural processes of infection, the defences come into play in the order—tissues, lymphatic system, blood stream, and finally reticulo-endothelial system. Given, then, an animal whose blood contains an adequate concentration of antibody specific for the antigens associated with the virulence of a parasite, we must consider whether the concentration of antibody in other tissues is sufficient for an effective antibacterial response at any of the stages of invasion we have listed. Serum antibodies, whether they result from active or passive immunization, are sooner or later distributed throughout the tissues. They are present in tissue fluids and lymph, but only in very low concentrations in cerebrospinal fluid (see Sohier and Jaulmes, 1939).

Thus Pagano (1894), Falloise (1903) and Batelli (1904) found that the concentration of hæmolysin was lower in the thoracic lymph than in the blood serum. Hughes and Carlson (1908), working with normal dogs, horses and cats, found that the concentration of hæmolysin for rabbit corpuscles formed a descending series, in the order—serum, thoracic lymph, neck lymph, pericardial fluid, aqueous humour. Becht and Greer (1910) studied the distribution of normal and immune antibodies in dogs. They also studied the rate of transference of antibodies from the circulating blood to the tissue fluids after passive immunization by the intravenous route. Their findings with regard to the distribution of normal and immune antibodies were in accord with those of Hughes and Carlson. In the actively immunized animals they observed a marked rise in the antibody titre of the serum, thoracic lymph and neck lymph, the relative concentrations in the different fluids maintaining the same order as in the normal animals. After passive immunization, by transfusion of blood from an immunized to a normal dog, the characteristic distribution of antibodies was established within $4\frac{1}{2}$ hours. These findings were confirmed in all essentials by Hektoen and Carlson (1910).

The thoracic duct, however, receives contributions from all the lymphatic channels of the body, including those from the liver, whose lymph ordinarily contains a high proportion of protein, so that the antibody content of thoracic duct lymph may reflect only the permeability of the liver capillaries to antibody. Thus Freund and Whitney (1929) injected agglutinins intravenously into rabbits and found that antibodies accumulated rapidly in liver lymph, slowly in lymph from the leg. Similarly, Becht and Luckhardt (1916) found cervical lymph had a lower concentration than thoracic duct lymph of intravenously injected antibody. In rabbits actively immunized with diphtheria toxoid, Takahashi (1936) found 0·67 unit of antitoxin per ml. of blood, and 0·29 unit per ml. of popliteal lymph. Nevertheless, with the possible exception of locally formed antibody (see Chapter 50) the concentration of antibody in tissue fluids and lymph, like that of other serum proteins, tends to be comparatively low. Thus in rabbits actively immunized with the virus of equine encephalomyelitis, the ratio of antibody titres in blood and brain tissue was found to be 120 : 1 (Morgan *et al.* 1942). In most cases the tissue antibodies are undetectable by direct titration of extracts (see, for example, Forattini 1937, Sedallian, Jourdan and Clavel 1938). With the onset of an inflammatory reaction, however, the greatly increased permeability of the capillaries and the lymphatics permits the exudation of antibody-containing plasma into the tissue spaces, and its drainage into the lymphatic channels. The concentration of antibody attained in the exudate varies with the nature and age of the inflammatory lesion (see, for example, Fox 1936) but in some cases it may equal that in the blood (see Finland 1932, Miles and Miles 1943).

The beneficial effects of this simple response may be reduced in a number of ways. The invading parasite may proliferate within the tissue cells (see Chapters 44 and 55) or it may be ingested by phagocytes. In both cases it will be protected from antibody

and other antibacterial substances in the blood. Thus Rous and Jones (1916) found that intraleucocytic typhoid bacilli were protected against antibody and against potassium cyanide; and similarly, Fothergill, Chandler and Dingle (1937) demonstrated that virulent influenza bacilli in the phagocytes of patients suffering from *H. influenzæ* meningitis were insusceptible to the action of specific antibacterial serum.

It is possible, too, that the capillaries may not permit the passage of the antibody molecule. The question arises particularly in the use of antipneumococcal horse sera, in which the antibody has a molecular weight of the order of 900,000, as compared with 150,000 for a normal serum globulin. It has been shown that rabbit antipneumococcal sera are more efficacious than horse antisera in experimental, and to some extent, in natural pneumococcal infections, and the effect has been ascribed in part to the greater penetrative power of the small rabbit antibody molecule (Horsfall, Goodner, McLeod and Harris 1937, Horsfall 1938). Haviland and McManus (1941), on the other hand, could find no difference in the diffusibility of rabbit and horse antibody into the inflamed pleural cavity of rabbits, but their inflammatory agent was a severe irritant and presumably induced maximum permeability in the blood capillaries.

The availability of tissue antibody is of paramount importance in the serum treatment of established infection. If exudation has already occurred in the inflamed region, then antibody introduced into the circulation will pass through the capillary walls with difficulty, owing to the hydrostatic pressure of the exudate already present in the tissues; in any event the antibody will be diluted in the existing exudate (Miles and Miles 1943); and the formation of extensive interstitial clots and intravascular thrombi (see Menkin above), or of dense and relatively avascular reacting tissue, may also prevent access of antibody to the infective lesion (see, for example, Kempf and Nungester 1939). Phenomena of this nature may also account in part for the therapeutic failure of antitoxin administered late in the course of a disease. In Chapter 46 we suggested that the failure might be due to a pharmacological inaccessibility of the toxin to antitoxin; but a mechanical inaccessibility is equally possible.

The availability of antibody in the tissues was studied along rather different lines by Friedemann and his colleagues. Friedemann and Zuger (1939*a*) observed that intradermal diphtheria toxin was fully neutralized in guinea-pigs already immunized with antitoxin, and that there was a linear relation between dose of toxin and dose of antitoxin. After the injection of toxin, neutralization could be achieved by the intravenous injection of antitoxin, but a comparatively much larger dose of antitoxin was required. The dose, however, was the same whether the antitoxin was given a few minutes after the injection of toxin, or up to four hours before; from which Friedemann and Zuger concluded that equilibrium between tissue and circulating antibody was reached a few moments after injection. Proceeding on this assumption, they attempted to measure the distribution of antibody between blood and tissue by comparing the concentration of antitoxin which neutralizes toxin on direct injection of the two into the tissue, with the blood concentration required to protect the tissue against a direct injection of toxin alone. In guinea-pigs the ratio of the two concentrations of antitoxin was about 20 for diphtheria and staphylococcal toxins given intradermally; in guinea-pigs and rabbits it varied between 50 and 1 for different batches of tetanus toxin given intracerebrally (Friedemann, Zuger and Hollander 1939, Friedemann and Zuger 1939*b*; see also Friedemann *et al.* 1944). It is recognized in this work that the ratios depend on the susceptibility of the animal tissue to the toxins, and variables of that nature, but the possible influence of changes in capillary permeability induced by the different toxins is not considered. Thus, the data supporting the hypothesis of a rapidly established equilibrium of antibody in blood and tissues are more readily explained on the assumption that the diphtheria toxin rendered the skin capillaries permeable for at least four hours, and, up

to that time, exudation of circulating antitoxins into the lesion was sufficient to suppress the typical toxic inflammation. Other workers find no evidence of a rapidly established distribution. Andersen (1937), for example, found that the antibody content of the skin of rabbits passively immunized against vaccinia by the intravenous route was relatively low after one day, and was equal to the blood content after 7 days.

These ratios, though they are not necessarily measures of the distribution of antibody between blood and tissues in the uninfected animal, are nevertheless indications of the magnitude of the antibody levels required in the blood of passively immunized animals for a protective effect in the tissues, irrespective of whether that protection is achieved by antitoxin already in the tissues, or by antitoxin exuding from the blood when the capillaries are damaged by the toxin. When we consider the greater complexity of factors concerned in action of tissue cells and fluids on bacteria, it will be clear that the blood levels necessary for the suppression of a tissue infection may be much higher (see also Fox 1943, Schlesinger, Olitsky and Morgan 1944).

The Availability of Antibody in the Lymphatic System.—Very little is known of the antibacterial effect of antibody in lymph We should expect that the lymph from an inflamed and infected region containing antibody would itself contain antibody and bacteria. Drinker and his colleagues (Drinker *et al.* 1935, Field *et al.* 1937) injected rabbits intravenously with virulent Type III pneumococci. Bacteria appeared, sometimes in large numbers, in the thoracic duct and in the cervical and leg lymphatics. The intravenous injection of moderate doses of antiserum was followed by a clearance of pneumococci from the blood, but not from the lymph; there was in some cases a small reduction of the bacterial content of the lymph when large doses of antibody were given. That the lymphatic system, which we have seen to be an integral part of the clearing mechanism of the body, should be demonstrably inaccessible to the sterilizing action of intravenously injected antibody, is a serious defect from the point of view of the serum therapy of infective disease, for bacteria in the lymphatic system will re-infect the blood stream as rapidly as it is sterilized, and vitiate the benefit conferred by the serum. The defect observed in Drinker's experiments may in part have been due to the relative impenetrability of the lymphatic system by the circulating antibody. Some antibody was found in the lymph of animals receiving large doses of antiserum, though not in very high titre. But even supposing that the antibody in the lymph was sufficient to sensitize any pneumococci present, it does not follow that the lymph would have been sterilized, since it contains few phagocytes capable of ingesting sensitized bacteria. For example, in heavily infected rabbits 88–95 per cent. of the lymph cells were lymphocytes, and no polymorphonuclear cells were seen. The defect, in fact, appears to be inherent in the animal, and not in the mode of serum therapy.

The Efficacy of Antigen-Antibody Union in vivo.—It must be remembered that the mere contact of antibody with bacteria does not necessarily ensure a maximum bactericidal effect. As we saw in Chapter 7, many *in vitro* antigen-antibody reactions take place with maximal effect only when the reactants are present in optimal proportions. In the therapy of natural infections by passive immunization, the only reactant we can vary, in quantity and quality, is antibody. It is not sufficient to assume that the largest practicable dose of antibody will necessarily be most successful.

Thus Goodner and Horsfall (1936) demonstrated that antipneumococcal horse antibody, which protected mice against virulent Type I pneumococci in a given dose, failed to protect in larger doses. In one experiment, pneumococci and serum were introduced into the peritoneal cavity of the mice. After four hours, in a mouse that had received

0·4 ml. of serum, there were about 215,000 extracellular pneumococci per ml. of exudate, and 24 per cent. of all the pneumococci present had been ingested by leucocytes; in a mouse receiving 0·05 ml. of the same serum, the corresponding figures were 690 extracellular pneumococci, with 97 per cent. of the total pneumococci ingested.

As in antitoxic immunity, the firmness of union between antigen and antibody is of considerable importance in bacterial immunity. Non-infective mixtures of bacteria and homologous antibody will become infective on simple dilution, indicating, as in analogous phenomena with "neutral" toxin-antitoxin mixtures, that some dissociation of the bacterium-antibody complex takes place. This dilution phenomenon was first demonstrated for bacteria by Enders and Shaffer (1937) and later by Morris (1940, 1941). The union appears to become firmer with the passage of time, for with prolonged contact of antibody and bacteria the dilute mixtures become less infective. Sabin (1935) found that mixtures of virus and antibody which were non-infective for one host given by one route might be infective when given by another route, or when given to another host, and postulated that the dissociation of antigen and antibody varied with the tissue and the animal. Morris (1944), taking fresh, complement-containing serum as a representative antibacterial tissue, tested its susceptibility to "infection" by *Salm. typhi*, with and without homologous antibody. Various specimens of fresh serum were alike in their susceptibility to bacilli alone, but varied greatly in susceptibility to mixtures of bacilli and antibody. She also observed two degrees of antibody action; a complete "neutralization," and, with less antibody, a modification of the bacilli that delayed their growth in the complement. The possibility of dissociation of antibody and infective bacterium, or of wide variations in susceptibility of tissues to "neutralized" and partly "neutralized" bacteria, has an obvious bearing on the problem of variations in the efficacy of antibody against bacteria in the different tissues and organs of the host, though at present we are largely ignorant of their significance in the elucidation of immune processes (see p. 1239).

Active Antibacterial Immunization as the Result of Infection.

We may close this chapter by considering what happens when a bacterium of moderate virulence obtains access to the tissues of a host that makes a successful immunizing response, and so recovers. We will take as an example a disease, such as typhoid fever, in which the bacterial parasite gains access to the blood stream and so is spread throughout the tissues during the early stages of the attack.

Fig. 247 shows diagrammatically the probable progress of an infection of this type. At A is represented the initial phase, during which the bacteria gain access through a mucous surface and pass to a regional lymphatic gland. At B the bacteria are proliferating in the gland, and there is an intermittent escape of some of them into the blood stream. At C these bacteria are shown as collecting in the liver and spleen, these organs being taken as examples of tissues rich in reticulo-endothelial cells, and hence concerned in the normal clearing mechanisms of the blood. At D the bacteria are proliferating in those situations in which they have collected during the initial phases of infection, which correspond to the incubation period of a clinical attack of disease. At E there is a re-invasion of the blood stream from these foci of bacterial proliferation, a well-marked bacteræmia, and a transport of bacteria to other tissues, not previously infected. This corresponds to the phase of clinical illness, and, in a fatal case, persists till death. Up to this stage no antibodies have appeared in the circulating blood; but at this point they usually make their appearance in cases that recover. At F is represented the phase of clinical recovery. The improved clearing mechanism, consequent on an efficient antibody-producing response, has terminated the bacteræmic stage; and we are left with a few scattered

foci of infection, in the spleen, liver and lymph nodes, and perhaps in other tissues. As we have seen, there is reason to believe that the persistence of this state of affairs over prolonged periods is consistent with the maintenance of apparent health. At G is represented the final stage of complete restitution, with the disappearance of all infecting bacteria, and the persistence in the tissues or body fluids of protective antibodies. It is probable that a proportion of infections may be terminated at

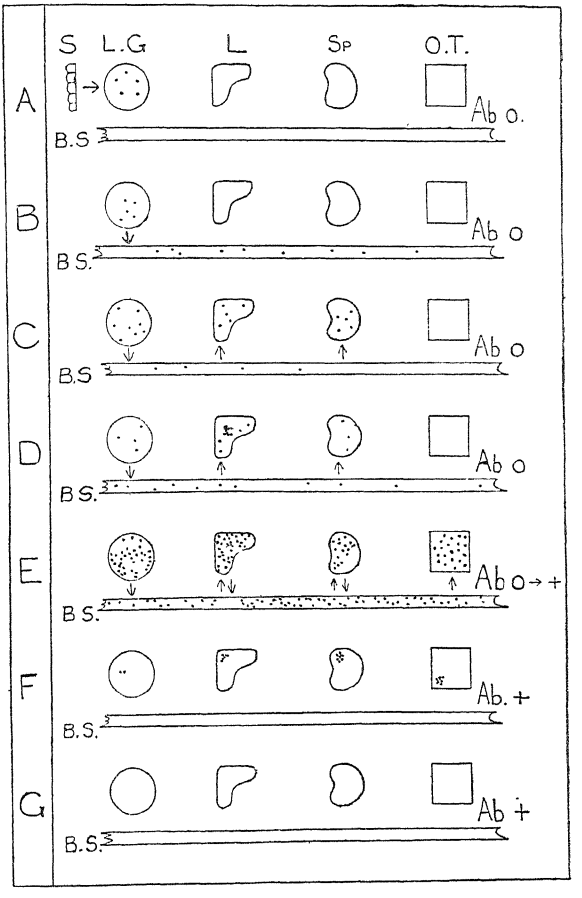


FIG. 247.

S = Mucous Surface. L.G. = Regional Lymphatic Glands. L = Liver. Sp. = Spleen. O.T. = Other Tissues. B.S. = Blood Stream. Ab.0. = Circulating Antibodies Absent. Ab. + = Circulating Antibodies present.

one of the initial stages, A, B, C or D, without ever passing to E, the stage of clinical illness; and there are reasons for believing that such abortive infections, especially if repeated, may lead to the acquirement of an effective degree of immunity to reinfection. With suitable modifications our diagram could be adapted to other bacterial infections, differing in regard to the primary focus of infection and the direction and extent of the secondary spread.

SUMMARY

(1) The mechanism brought into play in the removal of bacteria from the tissues is, in its main outlines, identical with that employed in the removal and storage of inert particles of the same order of size.

(2) When large numbers of bacteria are introduced directly into the blood stream they are in part taken up by the sessile histiocytes in such reticulo-endothelial depôts as the liver and the spleen, in part phagocytosed by polymorphonuclear cells and by macrophages in the lungs, in part flocculated into aggregates which are filtered off in the lung capillaries and later phagocytosed

(3) With avirulent bacteria, that have little power of multiplication within the tissues, this mechanism results in a rapid and permanent clearing of the circulation. With virulent bacteria an initial partial clearing is followed by a stage of bacterial multiplication in the foci to which the bacteria have been removed, resulting in a secondary invasion of the blood stream. The fate of the host is determined by the balance between this bacterial multiplication and the efficiency of the clearing mechanism. With slightly or moderately virulent strains there is a secondary bacteræmia followed by a secondary clearing. With highly virulent strains the secondary bacteræmia increases and the animal dies of an acute septicæmia. Even when secondary clearing occurs, and the animal remains in apparent health, foci of infection may remain in the various histiocyte depôts, and living bacteria may persist in these situations over long periods of time.

(4) When bacteria gain access to the body by other routes they are dealt with in part by local mechanisms of the same general character as that described under (3). The extent to which invasion of the blood stream occurs depends upon the anatomy and functional activity of the tissues first invaded, and upon the virulence of the invading organism. The blood stream is invaded very rapidly after intraperitoneal inoculation, less rapidly after intramuscular inoculation, and still less rapidly after subcutaneous inoculation. In all cases the passage from the primary site of infection to the blood stream is mainly by way of the lymphatics, and the histiocyte depôts in the regional lymphatic glands play a prominent part in the removal of the invading bacteria. When relatively avirulent bacteria gain access to the tissues at some site from which passage to the blood stream is slow, infection may never pass beyond the first line of defence in the regional lymphatic glands, but here, as in the case where a generalized infection has become established, localized foci of infection may persist over long periods of time.

(5) In addition to this process of removal by phagocytic cells, a direct bactericidal action of the serum plays a part in ridding the tissues of bacteria. The importance of this purely humoral immunity appears to differ widely in different bacterial infections ; but it is probably always subsidiary to the phagocytic mechanism, and is seldom, if ever, the main factor in defence.

(6) The efficiency of the clearing mechanism of a normal animal may be increased, *vis-à-vis* a particular bacterial parasite, by active immunization with a killed culture of that particular organism. The immunity so produced is specific in the serological sense. The actively immunized animal deals with a virulent strain of the bacterium against which it has been immunized in the same way as a normal animal deals with an avirulent or slightly virulent strain of the same bacterial species.

(7) Immunity of this type may be passively transferred by the inoculatio

into a normal animal of the serum obtained from an animal that has been actively immunized.

(8) The antibodies by which this passive immunity is conferred are those that unite with the surface antigens of the virulent bacteria; and in order to induce an effective active antibacterial immunity the immunizing agent employed must contain these surface antigens.

(9) The efficacy of humoral immunity in any part of the tissues is determined in the first place by the existing concentration of antibody on the cells or in the tissue fluid, and the availability of circulating antibody; and secondly by the presence, in optimal amounts, of the reactants, both fluid and cellular, necessary for the various antibacterial reactions of the tissues. It is probable that these factors vary widely in the different tissues of the animal body.

(10) The mechanisms described above come into play during the later stages of any bacterial infection that is associated with the development of an antibacterial immunity.

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CHAPTER 48

THE AGGRESSIVE ACTION OF BACTERIA

In the preceding chapters we have considered the ways in which bacteria injure the tissues of an animal host, and the ways in which the host defends itself against attack. In the present chapter we are concerned with certain bacterial products which, though not in themselves grossly toxic to the host's tissues, exert an aggressive effect in bacterial infections by interfering with the host's defence mechanisms.

The available data are, as is so commonly the case, sadly confused by the fact that we have been forced to work with the crudest and most complex of reagents. The easiest way of disentangling, to some extent, the confused threads of this particular problem is to take as our starting-point a series of observations recorded by Bail and his colleagues (Bail 1900, 1902–3, 1904*a*, *b*, *c*, 1905, Bail and Pettersson 1902, 1903, Bail and Weil 1906, 1911, Weil 1905*a*, *b*). Working with the anthrax bacillus, with organisms of the *Pasteurella* group, with the cholera vibrio and with the typhoid bacillus, they found that exudates produced in experimental animals by the inoculation of these bacteria had characteristic properties, which Bail attributed to the presence of special bacterial products to which he gave the name *aggressins*; these aggressins were, he believed, produced in the tissues of the infected host, but seldom, if ever, *in vitro*.

The properties of aggressins, as described by Bail, may be summarized as follows:

(1) Sub-lethal doses of bacteria are rendered lethal by the addition of aggressins.

(2) A lethal dose of bacteria, which alone would produce a slowly fatal infection, causes a rapidly fatal infection associated with characteristic lesions when aggressins are added to the inoculum.

(3) The addition of cholera aggressins neutralizes the bactericidal action of an anticholera serum, as tested by intraperitoneal injection in the guinea-pig.

(4) By the injection of aggressins an immunity is produced which is far more effective than that produced by the inoculation of bacteria and is different in kind. This immunity can be passively transferred.

According to Bail, the aggressins are not themselves toxic. They produce their effects, which are in the main specific, solely by aiding the rapid multiplication of bacteria within the tissues. He states also that they are extremely thermolabile, being completely inactivated by 30 minutes' exposure to a temperature of 55° C. and markedly affected by any temperature above 44° C.

Bail's contention, that the results of his experiments required for their interpretation the assumption of the existence of a hitherto unrecognized bacterial product of peculiar importance in infection and immunity and deserving the new title of "aggressin," did not long remain unchallenged. Wassermann and Citron (1907) recorded their reasons for believing that the aggressins were simply products

of bacterial growth, or of bacterial autolysis, which could be prepared in the test tube by suitable methods. Citron (1906) recorded experiments which showed that the addition of bacterial extracts to living bacteria increased their virulence; that by the use of such extracts it was possible to immunize animals against experimental infection; and that the serum of such immunized animals conferred passive immunity on others. Bail and Weil (1906) maintained that the immunity produced by Citron with his bacterial extracts was far less effective than that produced by natural aggressins, and that it was different in kind; though it is not entirely clear on what differential criteria they relied in distinguishing anti-aggressin immunity from the varieties previously recognized.

Doerr (1906) criticized the aggressin theory on somewhat similar grounds, but emphasized particularly the fact that large doses of dead bacilli may cause death in experimental animals with the lesions described by Bail as characteristic of the aggressin effect. He also noted that if sub-lethal doses of killed cultures were added to sub-lethal doses of living bacteria the combined inoculum might cause a fatal infection of the aggressive type. This additive effect did not appear to be in any way specific, but some killed cultures were more effective than others. For instance, dead typhoid or dysentery bacilli were more aggressive than dead staphylococci. In addition, Doerr demonstrated that aggressive exudates did, in fact, contain ordinary bacterial antigens; since a typhoid exudate gave well-marked precipitation with an ordinary antityphoid serum.

Sauerbeck (1907) has also criticized Bail's hypothesis. He concludes, from the results of a large series of experiments, that aggressive exudates are not lacking in toxicity, since two or three times the amount that produces the characteristic aggressive effect will kill an animal within a few days in the absence of living bacteria; that the activity of a given aggressive exudate is in no way proportional to the virulence of the organism that produced it, but rather to its toxigenicity; that characteristic aggressive effects can be produced with toxic bacterial products that have been prepared in the test tube; and that aggressive action, whatever its cause, is entirely non-specific. His findings as regards the heat-stability of the aggressive bacterial products also differ from Bail's, since he states that they are reduced in potency, but not completely inactivated by heating at temperatures between 55° C. and 70° C.

The detailed studies of Dudgeon (1912) on the properties of exudates obtained from natural infections in man and from experimental infections in animals made it clear that one of the mechanisms involved in the total aggressin effect was a specific anti-opsonic action.

The method he adopted in the majority of his experiments was as follows. He obtained exudates from natural infections in man or from experimental infections in animals. The exudate, after the separation of as much material as possible by centrifugation, was mixed in a capillary tube with an equal volume of normal serum, or of serum from an immunized man or animal. After 1 hour's incubation at 37° C. leucocytes and bacterial suspension were added to each mixture, the tubes were re-incubated for 15 minutes and films were prepared from the contents, the degree of phagocytosis being determined in the usual way. In control tubes the exudate was replaced by saline. In each case the opsonic effect of the serum, and of the serum-exudate mixture, was determined against the bacterium causing the infection and against some related organism. The results shown in Table 74 illustrate an effect that was commonly observed. The exudate came from a subcutaneous staphylococcal abscess; the immune serum was from the infected patient; the normal serum was from an uninfected control. The sera and the serum-exudate mixtures were tested against the strain of staphylococcus grown from the abscess and against a strain of *Bact. coli*.

TABLE 74

Mixture.	Number of Bacteria ingested by 50 Leucocytes.	
	Staph. aureus.	*Bact. coli.*
Normal Serum + Saline	240	229
Immune Serum + Saline	294	203
Normal Serum + Exudate	139	200
Immune Serum + Exudate	139	237
Exudate + Saline	61	21

The exudate alone had a slight opsonic effect, but it specifically reduced the opsonic action of normal or of immune serum on *Staph. aureus*, leaving unaffected their opsonic action on *Bact. coli*.

The substances responsible for this specific anti-opsonic effect were found, in many cases at least, to be markedly thermostable. Table 75 shows the effect of adding a boiled exudate, obtained from an experimental infection with *Salm. enteritidis*, to the serum of the infected animal.

TABLE 75

Mixture.	Number of Bacteria ingested by 50 Leucocytes.	
	Salm. enteritidis.	*Bact. coli.*
Serum + Saline	248	130
Serum + Boiled Exudate	27	102

In further experiments Dudgeon submitted exudates to boiling, removed the coagulum by centrifugation, added excess of alcohol to the clear supernatant fluid, collected the precipitate by centrifugation, and redissolved it in saline. The preparation so obtained still showed specific anti-opsonic action.

These results bore an obvious analogy to an earlier series of observations (Dudgeon, Panton and Wilson 1909–1910, 1910–1911). Extracts were prepared from various bacteria by grinding or by alternate freezing and thawing, and these extracts were shown to have a specific anti-opsonic effect. The anti-opsonic substances were markedly thermostable, remaining active after boiling for 30 minutes. A considerable series of control tests showed that they had no direct action on the leucocytes.

In addition to these heat-stable specific anti-opsonic substances, Dudgeon found that many infective exudates contained directly toxic substances, as judged by injection into experimental animals. In this respect, however, they varied widely, according to the infecting bacterium. It may be noted that certain highly toxic exudates, such as those obtained in experimental infections with *Salm. enteritidis*, retained their toxicity after boiling.

Further light has been thrown on the probable nature of the anti-opsonic substances in aggressive exudates during the prolonged and detailed study of the factors involved in antipneumococcal immunity.

Rosenow (1907) found that extracts or autolysates of virulent pneumococci had the power of inhibiting the phagocytosis of relatively avirulent pneumococci, and he suggested the name *virulins* for the active substances concerned.

Cole (1917) observed that empyema fluids from pneumococcal infections contained soluble substances which specifically neutralized the protective antibodies in an antipneumococcal serum, and that similar substances might be present in the blood of rabbits suffering from experimental pneumococcal infection. He showed that, when an antipneumococcal serum was injected intravenously into a rabbit previously infected with the same type of pneumococcus, the protective antibodies disappeared very rapidly from the blood, and that a similar phenomenon could be demonstrated in patients severely ill with lobar pneumonia.

The more recent studies on the antigenic components of bacterial cells that we have outlined in Chapter 8, and the demonstration that sensitization to the phagocytic action of polymorphonuclear cells and macrophages depends upon the union of antibody with an antigenic component situated at the surface of the bacterial cell, offer the obvious suggestion that the substances responsible for the specific anti-opsonic effects of aggressive exudates are dissolved bacterial antigens. Landsteiner's demonstration of the inhibition of antigen-antibody reactions by simple haptens (see pp. 256–258), and the *in vitro* reactions of specific bacterial polysaccharides that fail to stimulate antibody production *in vivo*, indicate that bacterial haptens, as well as complete antigens, may be expected to exert this anti-opsonic effect.

There is good evidence that this is actually the case. Sia (1926) has studied the action of the type-specific polysaccharides of Type II and Type III pneumococci on the growth of these organisms in serum-leucocyte mixtures from the rabbit and from the cat. The pneumococci were of relatively low virulence and developed very poorly in either of the serum-leucocyte mixtures employed. The addition of minute amounts of Type II polysaccharide greatly increased the growth of Type II pneumococci, but had little effect on the growth of the Type III organisms. The addition of minute amounts of Type III polysaccharide greatly increased the growth of Type III pneumococci, but not of Type II.

Felton and Bailey (1926) have demonstrated the aggressive action of Type II polysaccharide by injecting it into mice together with relatively avirulent pneumococci of the same type, and inducing a fatal infection. Type I polysaccharide had analogous effects (Ward 1930). Ward and Enders (1933) demonstrated that the anti-bactericidal effect was due to the specific inhibition of phagocytosis. The anti-phagocytic action of the Type I polysaccharide as prepared in the laboratory was not so pronounced as that of Type II and Type III polysaccharides. Nor was it as pronounced as that of extracts of pneumonic lungs infected with Type I pneumococcus (Ward 1932). The discrepancy was later resolved by the discovery that the polysaccharide preparations, unlike native polysaccharide, were not acetylated (see Chapter 8). Acetyl polysaccharide proved to be fully anti-opsonic (Enders and Wu 1934). The aggressive effect of the pneumococcal polysaccharide in human infections is suggested by the work of Cole (1917) and Park and Cooper (1928), who record that a fatal outcome in cases of lobar pneumonia in man is frequently associated with the presence in the blood of specific soluble antigen in amounts greater than can be neutralized by the antibodies that the patient has produced.

Downie (1937) observed an aggressin-like effect of Type I pneumococcal polysaccharide. Mice immunized with a single dose of polysaccharide were resistant to a given dose of Type I pneumococci. If this dose was injected together with 0·05 mgm. of the same polysaccharide, the immunity was in most cases abolished. Nye and Harris (1937) calculated that the specific polysaccharide present in the lungs of certain patients dead of pneumococcal pneumonia would have required more than 60 litres of a good antiserum to neutralize it. Frisch and his colleagues (1942) have found up to 0·033 gm. of polysaccharide per 100 gm. of lung tissue in areas of grey hepatization due to Types I, II, VII and VIII pneumococcus, and in 10 cases of Type III pneumococcal pneumonia an average of 1·57 gm. per 100 gm., which corresponded to a total lung content

of 68 5 gm. of specific polysaccharide in a fatal case. This amount is serologically equivalent to 750 gm. of antibody nitrogen ; even if we suppose that not all of it must be neutralized before some of the antibody is available for a direct antibacterial action, it represents a reservoir of " aggressin " in the lesion of sufficient size to account for the failure of serum therapy in Type III pneumonia (see Chapter 74).

The capsular substance of the pneumococcus, besides having an aggressive action in its soluble form, *i.e.*, combining in the tissues with antibody that otherwise would opsonize the living bacteria, is associated to some extent in the intact capsule with the virulence of the organism. This association appears to be due in part to the resistance of capsulated organisms to ingestion, either by phagocytes (Ward and Enders 1933) or by the reticulo-endothelial cells of the whole animal (Enders, Shaffer and Wu 1936). A similar relation between capsular substance and resistance to phagocytosis has been demonstrated in hæmolytic streptococci (Seastone 1934, Lyons and Ward 1935) ; and Lyons (1937) records capsulation in young cultures of *Staph. aureus* associated with a greater resistance to phagocytosis than that displayed by non-capsulated forms.

The destruction of capsular substance, or inhibition of its formation, by the action of enzymes renders bacteria more susceptible to the defence mechanisms. Thus, the enzyme specific for the polysaccharide of the Type III pneumococcus is protective against infection by virulent Type III pneumococcal infection (Avery and Dubos 1931, Goodner, Dubos and Avery 1932). The hyaluronic acid content of the polysaccharide material in certain Group A and Group C streptococci (Seastone 1939, 1943, Kendall, Heidelberger and Dawson 1938) renders them susceptible to the action of hyaluronidase (see Chapter 44). Hirst (1941) protected mice infected with Group C, but not those with Group A, by treatment with leech-extract hyaluronidase. Blundell (1942) found only a feeble protective action in bull-testicle hyaluronidase in Group A infections ; and McLean (1942) none at all in both Group A and C infections, with doses of enzyme in excess of those required for decapsulation of the streptococci *in vitro*. Kass and Seastone (1944), however, report that bull-testicle hyaluronidase increases the bactericidal power of normal blood for Group A streptococci, and will protect mice against Group A infection.

It is possible that the Vi antigen of *Salm. typhi* (see Chapter 30), which is associated with the virulence of the organism, may act analogously to the capsular substances of the cocci. It is only one-fiftieth as toxic as the O endotoxin (Henderson and Morgan 1938) ; nevertheless, intravenous injection of extracts of Vi bacilli greatly reduces the protective effect of Vi antisera in mice infected with the living bacilli (Henderson 1939, see also León and Morales 1943).

In addition to these dissolved haptens or antigens it is clear that some infective exudates will contain other more or less toxic substances of bacterial origin that interfere with the defence mechanism of the host. Both Pettersson (1940) and Stevenson and Reed (1940) record a negative chemiotactic effect in *Staph. aureus* ; the substances responsible appeared to be distinct from the recognized components of staphylococcal toxin. Pettersson's substance, which he calls " negatactic," was antigenic, and antisera prepared against it had a protective effect in guinea-pigs and mice. Pettersson later (1943) found similar negatactic substances, distinct from capsular substances, in *B. anthracis* and pneumococci. The anthrax anti-negatactic sera appeared to act by permitting the infiltration into the infected tissue of active phagocytes. The pneumococcal negatactic substances displayed some degree of host-specificity ; the phagocytes of man and of the rabbit were susceptible to its action, but those of the mouse and guinea-pig were not.

In some cases, the frank toxins of a bacterium have an undoubted specific action on the defence cells of the host. Two pathogenic species, at least, *Staph. aureus* and *Str. pyogenes*, produce a specific leucocidin that attacks the polymorphonuclear cells ; and, for example, Dennis and Senekjian (1939) report an antigenic leucocidin in *Salm. typhi*. We know little as yet with regard to the action of bacterial toxins on the cells of the reticulo-endothelial system ; but it is quite

probable that we shall find that certain bacterial species are capable of producing toxins that have a selective action on the macrophages.

There are also the bacterial coagulases, fibrinolysins and spreading factors, which have been included among the bacterial toxins. It is clear that, in so far as they play an active part in the genesis of infection, they might, perhaps with better justification, have been described as aggressins. It is possible, too, that bacterial products may have a direct destructive action on protective antibodies. Beumer (1938) records that certain toxigenic strains of *Staph. aureus* were able to produce toxin in broth containing antitoxin, a capacity they owed to the elaboration of a proteolytic enzyme which destroyed the antitoxin.

In summary : it would seem that we are justified in recognizing the following factors as involved, to a varying degree, in the defence rupture that is produced by the injection of sterile infective exudates, of dead bacteria, or of bacterial extracts, in association with non-lethal doses of living bacilli.

(1) *Dissolved bacterial haptens or antigens, or similar components still attached to bacterial protoplasm.* These act specifically by uniting with the corresponding antibody, and so preventing the effective sensitization of the living bacterial cells. Many of them are thermostable, and many are predominantly polysaccharide in nature. These haptens and antigens are usually associated with the virulent forms of the bacteria in question, and in some cases are demonstrably derived from the bacterial capsule. It is probably only those haptens or antigens that are situated at the surface of the bacterial cell that are able to function in this way; since it is the surface components that are concerned in sensitization.

(2) *Bacterial toxins that selectively attack the cells concerned in tissue defence.* Their action is usually non-specific *qua* the infecting bacterium, since the injury to the phagocytic cells will reduce the efficacy of the defence mechanisms against other types of invading bacteria. The nature of the attack on the tissue cells ranges from inhibition of phagocytosis to complete destruction of the cell. These substances may be either "endotoxins" or "exotoxins." Endotoxins of this type, if they characterize the antigenic surface of the bacteria, will clearly have a twofold aggressive effect in infective exudates; a specific neutralization of opsonic antibodies and a direct attack on the tissue cells.

(3) *Other toxic bacterial products, bacterial enzymes, fibrinolysins, etc.*, that may act in any of the ways that we have considered in Chapter 44. In many such instances the effect may be merely additive—the tissues may have to deal simultaneously with a toxæmia and an invasive infection which have no direct connection with one another. In other cases the bacterial toxins, enzymes or hormone-like products may interfere with the defence mechanism of the host in various ways. In any case such action is probably non-specific in the immunological sense, though it is quite likely that a particular bacterial product may favour one type of infection more than another.

To which of these bacterial products, if any, should we attach the label *aggressin?* There seems little advantage in using it at all; it describes an effect, not a substance. There is, perhaps, something to be said for the term *aggressive action*, if we can so define it as to make it fill a gap in our terminology. It would seem to be most usefully employed as indicating an action on the cells or body fluids that is not directly injurious, or of which the directly injurious effects are minimal, but which so interferes with the defence mechanism of the host as to favour, specifically or non-specifically, the multiplication of bacteria within the tissues.

Used in this sense, it may be compared with the term *defence rupture*, employed to describe the action of certain chemical agents such as calcium, silica, and benzene (see pp. 1206–1209).

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CHAPTER 49

THE NATURAL ANTIBODIES: THEIR NATURE, ORIGIN AND BEHAVIOUR

In the preceding chapters we have discussed the part played by antitoxins and antibacterial antibodies in the defence mechanisms of the host. We assumed, in that discussion, that these specific antibodies had been produced in response to artificial immunization or to natural infection. An adequate survey of any host species living under natural conditions will, however, show that many of the individuals composing it contain in their blood serum substances that react, *in vitro* and *in vivo*, in the same way as specifically induced agglutinins, or bactericidins, or antitoxins. The frequency of any particular antibody will be found to vary widely from one animal species to another, and often from group to group within the same species in accordance with age, or with some environmental circumstance.

The problems that concern us in this chapter are: (*a*) How do these natural antibodies arise? and (*b*) what part do they play in determining differences in natural immunity?

The Natural Antitoxins

The extensive evidence that we now possess with regard to the origin and significance of the natural antitoxins has, for the most part, been obtained in the study of two human infections, diphtheria and scarlet fever. It would, perhaps, be more natural to consider the relevant data in the chapters dealing with these two diseases; but it would be impossible to discuss our more general problem without frequent references to these particular instances, and it will therefore be more convenient to set out the evidence here.

That normal human beings may contain diphtheria antitoxin in their blood has long been known (Abel 1894, Wassermann 1895), but no extensive data on the distribution of antitoxin among the population at large were collected until after the description by Römer (1909) of the convenient intradermal technique for the measurement of small amounts of toxin and antitoxin. This was soon followed by the studies of Karasawa and Schick (1910), Schick (1911) and von Groer and Kassowitz (1915, 1917, 1919), which gave us a fairly detailed knowledge of the frequency of the presence of antitoxin among different age groups. The elaboration of the Schick test (Michiels and Schick 1913, Schick 1913), in which the presence of circulating antitoxin is detected by the insensitiveness of the skin to the intradermal injection of a small amount of toxin (the *negative* Schick reaction), has provided a still simpler method of detecting the presence of antitoxin above a certain level (1/100 to 1/30 unit of antitoxin per ml. of blood).

The proportion of persons who possess diphtheria antitoxin in the circulating blood varies in a striking and characteristic way as we pass from birth through

childhood and adolescence to maturity and old age. Table 76 sets out the extensive data recorded by Zingher (1923). It will be noted that the percentage of positive reactors is tabulated, *i.e.* the percentage that possess less than 0·01–0·03 units of antitoxin per ml. of blood.

At about 6 months of age just over half the infants tested react positively to the Schick test. The percentage of positive reactors then rises rapidly and between 8 months and 3 years fluctuates about an average value of approximately 88 per cent. After this period there is a continuous fall, at first rapid, then more gradual; between the 6th and 7th year the positive reactors number 50·4 per cent., by 12–13 they have fallen to 26·6 per cent., by 20–30 to 11·7 per cent., and at the allotted three-score years and ten to approximately 5 per cent.

TABLE 76

SHOWING PERCENTAGE OF SCHICK-POSITIVE REACTORS (*i.e.*, PERSONS CONTAINING LESS THAN 0·03 UNIT OF DIPHTHERIA ANTITOXIN PER ML. OF BLOOD) IN VARIOUS AGE GROUPS.

Age Group.	Number Tested.	Per cent Positive.
6–7 months	53	56·6
7–8 ,,	41	63·4
8–9 ,,	62	83 8
9–10 ,,	58	93·1
10–11 ,,	61	87·0
11–12 ,,	34	91·1
1–3 years	1,727	83·2
4–6 ,,	1,328	58 6
6–7 ,,	13,754	50 4
7–8 ,,	16,180	43 5
8–9 ,,	17,126	36·6
9–10 ,,	18,065	32·2
10–11 ,,	18,057	29 3
11–12 ,,	17,994	28·2
12–13 ,,	16,258	26·6
13–14 ,,	14,138	23·1
14–15 ,,	9,650	19·7
15–16 ,,	4,861	17·8
16–17 ,,	369	18·4
20–30 ,,	1,253	11·7
30–40 ,,	1,488	10·6
40–50 ,,	1,220	8·2
50–60 ,,	920	6 4
60–70 ,,	662	5·4
Over 70 ,,	181	5·5

The Origin of the Natural Antitoxins.

The most generally accepted view with regard to the succession of immunological events that underlie this observed fluctuation in skin-sensitivity to diphtheria toxin is as follows. At birth the majority of infants are endowed with circulating antitoxin which they have acquired passively from their mothers—mainly by passage of this antibody through the placental membrane, in part perhaps with the colostrum in the early days of suckling. This passive congenital immunity, like all other types depending on the passive acquirement of antibodies, will be relatively short-lived. A careful study of the disappearance of antitoxin from the blood of an infant whose mother was highly immune is recorded by Neill and his colleagues (1932). From infancy onwards the child will be exposed to the

risk of infection, increasing from year to year during the earlier part of school life. The result of this exposure will be that some children will contract the typical disease, while others will escape with mild and atypical infections, or with infections so slight that they fall wholly within the bacteriological as opposed to the clinical sphere. Each of these infections will stimulate the production of antitoxin, so that there will result a gradual process of natural immunization, the transitory passive immunity of early infancy being thus replaced, after a period of susceptibility, by a lasting active immunity.

This description, it should be noted, is not based on the results of Schick testing alone ; it is supported by a considerable body of evidence. Observations carried out by Römers' technique, and particularly the extensive series of observations made by von Groer and Kassowitz (1915), have shown clearly that the results of the skin test afford a reliable general indication of the frequency of circulating antitoxin in the different age groups. Thus 84 per cent. of infants at birth were found to have diphtheria antitoxin in their blood in a concentration of 1/200 A.U. or over, at ages 9–18 months the proportion had fallen to 32 per cent. and by 2½–3½ years to 28 per cent. It then rose steeply and by 17–18 years was back at the birth figure of 84 per cent. Later work has confirmed this picture, particularly the increasing proportion of Schick-negative persons with increasing age. The antitoxin blood level associated with the Schick-negative state, however, is not constant, some persons being positive with over 0 05 A.U. per ml. of blood, others negative with less than 0·005 A.U. per ml. (see Jensen 1933, Parish and Wright 1938).

There is, moreover, direct evidence that antitoxic immunity to diphtheria in infants results from transference of antitoxin from mother to child. Von Groer and Kassowitz tested the blood of 143 mothers and their new-born children. In 96 per cent. of cases antitoxin was present in both mother and child, or absent in both. Wright and Clark (1944), summarizing their own investigations and those of four other observers, found agreement in the Schick test of mothers and their infants in 84–85 per cent. of 713 tests.

Additional evidence of the transference of maternal antitoxins is provided by the observations of high antitoxin levels in the blood of infants whose mothers were immunized during pregnancy (see Magara 1936, Liebling, Youmans and Schmitz 1941), though in this case the possibility of a direct antigenic stimulus to the fœtus is less remote than in the cases cited above. In some instances discrepancies have been noted between the result of the skin test in infancy and the presence of antitoxin in the blood, a proportion of Schick-negative infants showing little if any circulating antitoxin (Kellogg 1926–7, Friedberger and Heim 1929, Okell 1932, see also Cooke and Sharma 1932). The Schick-negative state may in some cases be due to an insensitivity of the infants' skins that is independent of the presence of antitoxin (Dudley, May and O'Flynn 1934).

We may note here, although it concerns antibacterial antibodies, that the blood of infants of mothers actively immunized with *H. pertussis* vaccine contains a high level of protective antibodies against experimental *H. pertussis* infections (Cohen and Scadron 1943).

The mechanism of passive congenital immunity has been studied experimentally in animals. Ehrlich (1892), for instance, demonstrated the transference of anti-abrin and antiricin from female mice to their young by both the transplacental and mammary routes. It would seem that the relative importance of the two routes of transference depends upon the number of tissue layers that separate the maternal and fœtal circulation in different animal species. Table 77, taken from a paper by Mason, Dalling and Gordon (1930) sets out this relation in summary form.

Schneider and Szathmáry (1938, 1939, 1940) classified mammals according to the presence or absence of four out of a total of seven tissue layers between maternal and fœtal circulation. The results of their investigations with regard to the transmission of diphtheria antitoxin and agglutinins for *Salm. typhi* from maternal to fœtal blood are, however, essentially similar to those in the table, except that in dogs (*i.e.*, a carnivore) transmission *via* colostrum was marked, and placental transmission negligible.

TABLE 77

THE RELATIVE IMPORTANCE OF THE PLACENTAL AND MAMMARY ROUTES IN THE PASSIVE TRANSMISSION OF ANTIBODIES FROM MOTHER TO OFFSPRING. (After Mason, Dalling and Gordon.)

Species	Layers of Tissue between Maternal and Fœtal Circulation	Importance of Placental Transmission.	Importance of Transmission by Colostrum
Pig	5	−	+++
Ruminants	4	−	+++
Carnivores	2	±	+
Rodents, apes, man	1	+++	±

In man, antibody is present in the colostrum and milk, though usually in small amounts, unless the blood antibody levels are high. Immediately after delivery, for example, the diphtheria antitoxin content of the milk may be as high as one-third that in the blood, but it drops steeply during the first few days of lactation. Nevertheless, some transmission *via* the milk appears to take place (Sugg 1935, Magara 1936). Mice differ from other rodents in the table in that the mammary route appears in some cases to be more important than the placental. Thus, a higher degree of milk transmission of antibodies has been demonstrated experimentally in mice infected with herpes virus (Berry and Slavin 1943) and with *Trypanosoma duttoni* (Culbertson 1940).

Before considering the significance of the findings in the later age groups we may note that the distribution of scarlatinal antitoxin among the different age groups, as judged by the Dick test, appears to be essentially similar to that of diphtheria antitoxin. Table 78 gives the results obtained by Zingher (1924) in testing 7,700 persons.

TABLE 78

PERCENTAGE OF POSITIVE DICK REACTIONS IN DIFFERENT AGE GROUPS (ZINGHER).

Age Group.	Number of Persons Tested.	Per cent. Positive.
0–6 months	29	44·8
6–12 „	52	65·3
1–2 years	233	71 6
2–3 „	204	64·2
3–4 „	241	60·5
4–5 „	264	48·4
5–10 „	1,955	33·6
10–15 „	2,965	22·8
15–20 „	981	16·8
Over 20 „	776	14·4

It would seem that a negative Dick test during the first few months of life is as likely to be due to an insusceptibility of the infant skin to scarlatinal toxin as to the presence of antitoxin in the blood and tissue fluids. Cooke and others (1927, 1928) tested 200 mothers and their offspring. The results confirmed the rarity of positive Dick reactions in new-born infants; but the mothers of some of the negatively-reacting infants reacted positively, and some of the infants had no detectable antitoxin in their blood. When tested six weeks to three months later these infants were usually found to have become Dick-positive, while later still they became Dick-negative again. This reversion to the Dick-negative state was always associated with the appearance of antitoxin in the circulating blood.

A number of workers have recorded antistreptolysin in high titre in the blood of newborn infants, apparently derived from the mother. Though its significance is not clear it should be noted that these infant titres are sometimes found to be consistently higher than those in the maternal blood (see Plummer 1938, Gordon and Janney 1941).

Returning to the course of events from infancy onwards, are we justified in accepting the view that the acquirement of antitoxic immunity with increasing age is entirely due to natural active immunization resulting from the reception from without of specific stimuli in the shape of clinical or subclinical infections? This hypothesis has certainly not been placed beyond dispute. It has indeed been vigorously disputed, particularly by Hirszfeld (1926) and his colleagues.

Hirszfeld believes that all the normal antibodies should be regarded as "biochemical organs," with which the individual is endowed as the result of his evolutionary history. These "biochemical organs" ripen, or come to maturity, at different periods in his own individual development; just as the various organs and tissues of the body come to full maturity at different ages. As we speak of a "morphogenesis" through which each individual passes, so we should think of a "serogenesis" which forms an integral part of normal development. The time at which any particular antibody normally makes its appearance may be regarded as the period of "immunological crisis" for that particular antibody, and marks the establishment of a "biochemical reflex" on which the production of that antibody depends. Until the biochemical reflex has become established attempts at immunization with the particular antigen concerned will have little effect. Hirszfeld does not deny that specific, or non-specific, stimuli from without may play some part in accelerating the production of particular antibodies, or in increasing their concentration; but he would relegate such influences to a very secondary place. He would not regard the presence in the blood stream of antibodies acting on a particular bacterium as valid evidence of past or present infection with the bacterium in question. Above all, he would not accept the view that the antibody-forming apparatus is a *tabula rasa*, on which environmental stimuli can write at will. It is a complex mechanism with its possible activities fixed and ordered by the phylogeny of each animal species; and it will therefore respond to the appropriate stimuli and to no others.

It will be simpler to discuss the more general aspects of Hirszfeld's views when we have dealt with the origin of antibacterial sensitizers, and to confine our immediate attention to antitoxic immunity, noting in passing that the problem is in no sense a purely academic one but has important bearings on many problems of diagnosis and prevention.

Perhaps the most significant data, supporting Hirszfeld's contention, are to be found in observations (Hirszfeld, Hirszfeld and Brokman 1924, Hirszfeld and Hirszfeld 1927) which suggest a linkage between the inheritance of the power to produce antitoxin and the power to produce the iso-agglutinins which determine the human blood-groups (see p. 1087). From these records Hirszfeld draws the following conclusions. When both parents are Schick-negative the children will be Schick-negative, except during the first few years of life. When both parents are Schick-positive the children will be Schick-positive, and will remain so throughout life. When one parent is Schick-negative and the other Schick-positive the children who belong to the same blood-group as the Schick-negative parent tend to be Schick-negative, those who belong to the same blood-group as the Schick-positive parent tend to be Schick-positive (see also Tamaki *et al.* 1936). The validity of these observations has, however, been questioned by Rosling (1928), who examined 50 children over one year of age whose parents belonged to different blood-groups and reacted differently to the Schick test. He found that 23 children gave the same Schick reaction as the parent to whose blood-group they belonged, while 27 reacted differently.

A negative is notoriously difficult to prove; and we cannot assert that no child has ever developed diphtheria antitoxin except in response to the specific stimulus

provided by diphtheria toxin. We can, however, study the frequency of negative and positive Schick reactors in different samples of the population, and correlate our findings with the history of exposure to infection, so far as such a history is obtainable. If we find that free or frequent exposure to infection is consistently associated with a high percentage of negative reactors, while population groups that have been relatively free from any risk of infection show a notably low percentage of negative reactors, we shall be inclined to attach great importance to the part played by environmental stimuli, and to be a little sceptical as to the occurrence of spontaneous antitoxin production. The same holds, of course, in antiscarlatinal immunity and the Dick test.

Zingher (1923) noted that the proportion of Schick-positive children in different New York schools fluctuated very widely (14·62–70·13 per cent.), and that the schools with the highest percentage of positive reactors were those attended by the children of the more fortunate classes, who would have been less likely to have contracted the infection during early life, while a low percentage of positive reactors was found in schools attended by children of the poorer classes, and particularly in orphan asylums. The effect of institutionalism on the frequency of the Dick reaction is similar. Table 79 gives a series of observations recorded by Dyer and others (1926). Anderson (1943) reports a striking variation in the percentages of Schick-positive children according to their past experience of the disease : in the course of an extensive survey he found that 30·6 per cent. of children with a history of diphtheria, and 26·6 per cent. of children with a history of contact with diphtheria patients, were Schick-positive, while the corresponding rate in children with no history of the disease or contact with it was 90·9 per cent.

TABLE 79

PERCENTAGE OF DICK-POSITIVE CHILDREN IN CERTAIN SCHOOLS IN WASHINGTON. (After Dyer, Caton and Sockrider.)

School.	Age in Years.	No. Tested.	Per cent. Positive.
Suburban	3–15	75	77 3
Suburban	6–15	59	74·5
33 Rural Schools	4–21	1,147	72·5
33 Rural Schools	6–15	1,039	73·6
Episcopal Home	6–15	47	38 3
Orphan Asylum	3–14	80	32·5
Orphan Asylum	5–15	148	8·1

We might hope to obtain data of the same kind by comparing the frequency of Schick-positive reactions in countries where diphtheria is common with their frequency in countries where it is rare ; but the presence or absence of a particular disease among native races, and especially among their children, is less easy to determine than might be supposed, and such evidence as we have is conflicting.

Smits (1926) states that diphtheria is a very rare disease among the natives in the Dutch Indies, and it might therefore be expected that a high proportion of the native population would give a positive Schick reaction. This, he found, was not the case. Among 600 Javanese natives positive results were obtained in only 2 per cent. of the men and 8 per cent. of the women, while 41 per cent. of the children reacted positively. If the statement of the rarity of diphtheria could be accepted, this striking decrease in the proportions of Schick-positives with age would clearly support the view that antitoxin may be elaborated in the absence of any external specific stimulus. Kirschner (1929), however, records a detailed study of the prevalence of diphtheria in the Preanger district of Java during the period 1921–27, and notes the great increase in the number of recorded

cases which followed the provision of facilities for bacteriological diagnosis in 1923. He produces evidence that the disease is an important cause of mortality among native children and that a mild form is relatively common. We cannot, therefore, accept the findings with regard to the Schick test as supplying valid evidence in support of Hirszfeld's views.

Smits' observations do not stand alone. Heinbecker and Irvine-Jones (1928) performed Schick tests on 49 Eskimos; only 12 gave positive reactions, and of these 11 were under 12 years of age. Antitoxin was demonstrated in some of the negative reactors. Yet diphtheria is said to be unknown among the Eskimos.

Kleine and Kroó (1930) tested 101 East African natives by the Schick reaction; 95 of these were children between the ages of 6 and 15, the remaining 6 were adults. In no case was a positive reaction obtained. The toxin employed was subsequently retested and found to have lost none of its activity. Specimens of serum were obtained from 11 of the natives and tested for antitoxin; 10 of these contained antitoxin in considerable amount (0·05 A.U. to more than 1 A.U. per ml.). It is clear therefore that the East African native frequently forms diphtheria antitoxin early in life; yet, according to Kleine and Kroó, this population is not, so far as is known, exposed to infection with diphtheria. (See also Parr and Avery 1926–27, Parr, Goodale and Kirschner 1930.)

Grasset and his colleagues (1933) record a high frequency of natural diphtheria immunity among the Bantu of South Africa, as judged both by Schick tests and antitoxin titrations. They note, however, the occurrence of diphtheria among the populations concerned, and adopt without question the view that the antitoxin has been produced in response to infection. Murray (1943) provides more definite evidence. Among the Johannesburg Bantu clinical diphtheria was rare, but, as Grasset found in his series, the Schick-positive rate was low. In rural school children it was 8 0 per cent. and in urban children 13·7 per cent. The corresponding carrier rates of virulent diphtheria bacilli were 1·8 per cent. in the urban, and 3·2 per cent. in the rural; and though the Schick-positive rates and the carriers did not vary together, immunization was associated with the presence of carriers among the children. It is noteworthy that there was little clinical diphtheria among these Bantu children, as compared with white children in the same district, among whom a similar carrier rate was associated only with an epidemic of diphtheria; no bacilli were found in their throats in non-epidemic periods. Murray (1942) concludes that environmental conditions were in part responsible for the high level of antitoxic immunity of the Bantu, and that the absence of clinical diphtheria in spite of the relatively high carrier rate was due to an ability, perhaps racially determined, to produce antitoxin quickly in response to the specific antigenic stimulus (see Black 1934).

In only a few other instances do such surveys include a record of the carrier rate of diphtheria bacilli, or of hæmolytic streptococci, among the population concerned. Wells (1933), records a study of the Schick reactions of central and polar Eskimos, in which a proportion of tests were confirmed by antitoxin titrations, and throat swabs were obtained from all persons examined. He reports the frequent presence of bacilli morphologically resembling *C. diphtheriæ*, and states that four strains gave the typical fermentation reactions, and one strain showed some degree of virulence. These findings clearly tend to throw doubt on the significance of the observations recorded by Heinbecker and Irvine-Jones (1928), but it can hardly be said that the presence of toxigenic diphtheria bacilli was established with certainty.

A study by Asbelew and Margo (1932) of the Schick and Dick reactions of 103 of the 250 inhabitants of the Arctic island of Kolgujew is very difficult to reconcile with the *Durchseuchung* hypothesis—the view that antitoxin is produced only or mainly as the result of infection. With the exception of one girl of 8 years all persons examined were Schick-negative, and with the exception of one doubtful case all were Dick-negative. The antitoxin content of the blood was unfortunately not determined. Diphtheria and scarlet fever had never been reported on the island. Swabs were obtained from the throats of 168 persons, and from the noses of 93. No diphtheria bacilli were isolated, and hæmolytic streptococci were found in only four swabs.

There is great need of further studies in which full bacteriological and immunological tests are carried out on isolated or semi-isolated populations.

Although it is not complete, in this particular sense, the observations of Dungal (1932) on diphtheria in Reykjavik are particularly instructive. From 1926 onwards diphtheria has been very rare, not only in Reykjavik but in Iceland as a whole; but in the preceding years there was a moderate prevalence, reaching its peak in Reykjavik in 1921 with an attack rate of 6·9 per 1,000, and subsiding rapidly during the following 5 years. In December 1931 and during the early months of 1932 Dungal tested 814 school children in Reykjavik between the ages of 8 and 14 years. Of these, 81·9 per cent. gave a positive Schick reaction, a figure differing widely from that recorded in cities such as New York or London, where diphtheria is endemic. Still more significant, the curve describing the development of Schick immunity with age was of a very different kind. There was a trivial rise in the proportion of negative reactors from 13·97 per cent. in the 8-year age group to 17·24 per cent. in the 11-year age group; but there was a sudden rise to 26·36 per cent. among the 12-year-olds, and a further rise to 37·50 per cent. at 13 years. This clearly corresponds with the inclusion of children who had experienced the epidemic prevalence of 1921 and the

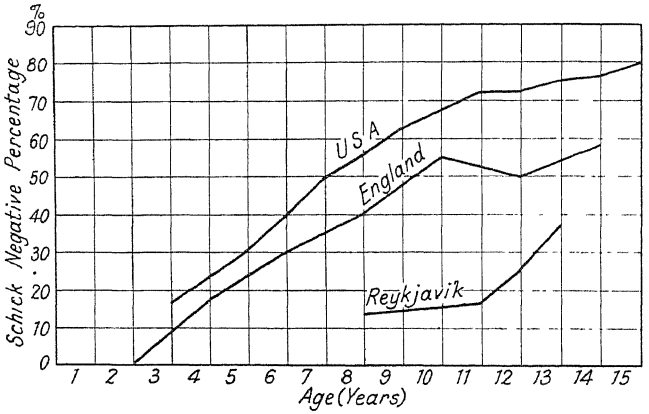


FIG. 248.

neighbouring years. Fig. 248, reproduced from Dungal's paper, illustrates these facts very plainly. Such an experience as this is clearly a weighty piece of evidence in favour of the dominant rôle played by latent infection in the stimulation of antitoxin production.

The semi-isolated population of Reykjavik provides yet another indication of the rôle of latent infection. Sigurjónsson (1939) noted that the duration of immunity following the immunization of children in the 7–13 year age group, estimated by the percentage of reversions from the Schick-negative to the Schick-positive rate during a four-year period, was much shorter than that in European children, though similar antigens were used in both cases. The European figures, however, were obtained from communities in which diphtheria was prevalent, whereas there was no clinical diphtheria in Reykjavik during the period of observation. The European immunity, it appears, was maintained by the continued stimulus of latent infections; in Reykjavik, where the risk of latent infection was minimal, the immunity declined more quickly.

One point of interest arises in the association of antitoxic immunity with carrier rates in a community, namely, the relation of avirulent strains of *C. diphtheriæ* to the immune state. By definition (see Chapter 61) the avirulent bacillus is non-toxigenic, and presumably is incapable of inducing an *antitoxic* immunity. The ratio of avirulent to virulent strains isolated varies from one community to another. Emmerson (1941) records a ratio as high as 13·5 : 1 in South Africa, and Forbes (1932) a ratio as low as 1 : 2 in London.

The lower ratios are usually found in white children living in relatively temperate conditions (Chason 1936, Gill 1940, Grossman 1940) and the higher with tropical conditions (Vardon 1923, Gomez and Navarro 1923); though Murray (1943) found ratios of 1 : 1 and 2 5 : 1 in South African Bantu, while Stebbins (1940) in New York State records 23 avirulent and no virulent strains in 1,800 children, of whom one-third were Schick-negative. We may regard these strains either as capable of producing minute but immunizing amounts of toxin and consequently of inducing a Schick-negative state in the carrier; or, as the experiments of Frobisher and Parsons (1943) on the immunization of rabbits with avirulent strains suggest, as inducing a useful anti-endotoxic immunity which may indirectly stimulate the increased production of antitoxin in subjects subsequently exposed to toxigenic bacilli. The high proportion of avirulents in some communities may, as Dudley (1929) has suggested, indicate a close adaptation between *C. diphtheriæ* and a population with a high level of immunity.

Taking the evidence from isolated, or semi-isolated, populations as a whole it can hardly be said to tell decisively for, or against, the *Durchseuchung* hypothesis. Its balance will depend, for each of us, on the particular records that inspire us with most confidence.

Another very instructive way in which we may obtain evidence with regard to the influence of environment on antitoxin production is to study *the rate of change* from the Schick-positive to the Schick-negative state under different conditions.

A good example is given by Zingher (1923), who records the percentage of Schick-positive children in different age groups among the inmates of a particular institution, dividing them into those who had been resident there for more or less than 3 months. The figures are set out in Table 80. The numbers examined in some of the groups were small so that the corresponding percentages must be accepted with caution; but there can be no doubt as to the significance of the marked difference between the recently admitted inmates and the older inhabitants. The institutional environment had clearly induced a swing from positive to negative reactiveness, with a velocity far higher than could be accounted for by changing age.

TABLE 80

PERCENTAGE OF CHILDREN GIVING POSITIVE SCHICK REACTIONS AMONG THOSE WHO HAD RESIDED IN A CERTAIN INSTITUTION, (*a*) FOR MORE THAN 3 MONTHS, AND (*b*) FOR LESS THAN 3 MONTHS, ACCORDING TO AGE GROUP. (After Zingher.)

Age Group.	Over 3 Months.	Under 3 Months.
4–5 years	23·8	66·6
5–6 ,,	27·3	33·3
6–7 ,,	17·0	43·0
7–8 ,,	13·9	43·7
8–9 ,,	4·6	33·3
9–10 ,,	3·8	9·0
10–11 ,,	1·6	33·3
11–12 ,,	5·0	25·0

Perhaps the most conclusive evidence of the effect of environment and infection is provided by such studies as those of Dudley (1923, 1926), who observed a small semi-isolated community of boys through a period of several years during which it was visited by three waves of diphtheria. By testing the Schick reactions at different periods he was able to demonstrate a wave of antitoxic immunization associated with each wave of disease.

During an extensive study of the relation of Dick reactivity to the prevalence of scarlet fever in Roumania, Schwentker, Janney and Gordon (1943) could find little evidence that

the acquisition of *Str. pyogenes* in the throat, or of infection with *Str. pyogenes* other than clinical scarlet fever, had any effect on the rate of change from the Dick-positive to the Dick-negative state. A similar rate of change, characterized by a slow increase in the earlier age groups, and a rapid increase with increasing age up to 20 years, was observed in a number of village communities, irrespective of the presence of scarlet fever (though in some cases an epidemic was followed by a general diminution in the percentage of negative reactors) and of other streptococcal infections. There was clearly an association between maturity and the acquisition of the Dick-negative state, but it was not clear how far latent infections could be altogether excluded as a contributory factor.

There is no doubt at all that the frequency of Schick and Dick reactors in any population or community in which diphtheria and scarlet fever are prevalent is determined very largely by the degree of exposure to infection ; and active immunization certainly plays a large part in natural antitoxin production under such conditions. It seems unlikely that antitoxin would be produced in the entire absence of such specific stimuli ; but we are not in a position to deny this possibility.

It is of some interest to note that numerous observers have recorded the presence of diphtheria antitoxin in the blood of normal horses. Sordelli (1920) found antitoxin in the blood of horses under 9 months old, presumably resulting from passive congenital immunization. No horse between 1 and 5 years of age possessed antitoxin, but the blood of all horses over 10 years of age contained detectable amounts. Glenny (1925) found 0·1 A.U. or more per ml. in 70 per cent. of horses over 7 years of age tested between the years 1907 and 1915, but in only 35 per cent. of those tested after 1917. He suggests that this difference may be due to the greatly lessened concentration of horses in towns during the later period, and a resulting diminution in the risk of infection. Lemetayer and de Diétrich (1936) record that no antitoxin was found in the blood of horses kept mainly at grass in a stud farm, whereas a fair proportion of stabled horses in surrounding farms, and from 50–70 per cent. of military horses, were positive. Ramon, Richou and others (1936) studied a number of immunized horses, and found that antitoxins were present only for those toxins of which the animals might reasonably be supposed to have had experience ; *i.e.*, the tests were positive for antitoxins of *C. ovis*, *C. diphtheriæ* and *Staph. aureus*, but not for anti-abrin or for the antitoxin to viper venom. A few well-documented instances are on record showing that horses are liable to natural infection with diphtheria bacilli. In most cases the organisms have been isolated from superficial wounds (Minett 1922, Kliewe and Westhues 1925, Parish and Okell 1926) ; only in a few instances have they been demonstrated in the throat (Cobbett 1899, Ramon 1925, Zuruk-zoglu and Mündel 1935*a*, *b*). We have, however, no adequate evidence with regard to the frequency of such infections, so that we cannot tell whether the presence of antitoxin in the blood, as recorded by Glenny, can reasonably be attributed to external stimuli of this kind.

Natural staphylococcal antitoxin is found in the blood of guinea-pigs. The rôle of exposure to environment in the stimulation of antitoxin was studied by Ramon and Richou (1936), who found that 77 per cent. of guinea-pigs kept in stables had a serum-content between 0·1 and 20 international units per ml. ; while the corresponding figure for guinea-pigs kept in open fields was 5 per cent. They later showed that guinea-pigs, kept in contact with guinea-pigs with a high natural antitoxin content, themselves developed a high content, in contrast to segregated control guinea-pigs, which developed no antitoxin (Ramon, Richou and Djourichitch 1936).

The Immunological Significance of the Natural Antitoxins.

Whatever the mechanism of their production, there is no doubt that the natural antitoxins confer on their possessors a significant degree of immunity. Direct experimental evidence is, of necessity, scanty ; but so far as it goes it is demonstrative.

Guthrie, Marshall and Moss (1921) collected 8 volunteers and determined their reaction to the Schick test; 4 reacted positively and 4 negatively (including 2 pseudo-reactions). The throat of each of these 8 volunteers was swabbed with a virulent culture of the diphtheria bacillus. The four positive reactors developed clinical diphtheria; the 4 negative reactors, possessing natural antitoxin at or above the Schick-immune level, developed no illness, though 3 of them became carriers.

The main body of evidence on which we rely in concluding that natural antitoxin confers natural immunity has, however, been collected during the course of extensive clinical and epidemiological studies on diphtheria and scarlet fever (see Chapters 61 and 66). There is no doubt that a person whose blood contains antitoxin at or above the Schick-immunity level is far less likely to contract diphtheria than a Schick-positive person. If he does so, the attack will often be mild and will seldom prove fatal. The nature of the evidence that associates natural antitoxin immunity with effective resistance to scarlatinal infection is of the same general kind as in the case of diphtheria.

The Natural Antibacterial Antibodies

It has long been known that there are naturally occurring agglutinins, bactericidins and other sensitizing antibodies, just as there are naturally occurring antitoxins; but the ground that has been surveyed in the two cases is rather different. We have seen that our knowledge of the distribution and mode of origin of the natural antitoxins is, in the main, derived from the study of diphtheria and scarlet fever in man. Our knowledge of the distribution and mode of origin of the antibacterial sensitizers is, in the main, derived from studies on their occurrence in different species of animals.

One of the earliest studies of this kind was recorded by Burgi (1907). He tested sera from the dog, guinea-pig, rabbit, goose, hen, sheep, goat, horse, ox and man against suspensions of various bacteria, including *V. cholerœ*, *Salm. typhi*, *Bact. coli*, *Past. aviseptica*, *Staph. aureus* and *Proteus vulgaris*, and noted well-marked agglutination in many instances, often to a relatively high titre. He found, however, that the various sera tended to be arranged in very much the same order of activity, against whichever species of organism they were tested; and, in further experiments, that this order was in general maintained when the sera were tested for their ability to flocculate suspensions of mastic.

Gibson (1930) carried out similar experiments with sera from the ox, rabbit, guinea-pig, horse, sheep, pig, rat, cat and man, tested against *Proteus X*19, *Ps. pyocyanea*, *Salm. typhi*, *Salm. paratyphi A*, *Salm. paratyphi B*, *Salm. enteritidis*, *Sh. flexneri*, *Sh. shigœ*, *Proteus morgani*, *Bact. friedländeri*, several strains of *Bact. coli*, and *V. cholerœ*. He found, as Bürgi had done, that the sera tested tended to fall in a definite order with regard to their power of agglutinating a wide variety of bacteria. Ox sera were the most active; pig and horse sera were somewhat less active; sheep serum was next in order; while human, cat, rabbit and guinea-pig sera, in that succession, showed progressively weaker effects. Rat serum was the weakest of all. Similarly the different bacterial species showed a definite order of sensitivity to agglutination. *Sh. flexneri*, *Bact. friedlanderi* and *Ps. pyocyanea* were the most sensitive, while certain strains of *Bact. coli* were least sensitive. By absorption experiments, Gibson was able to show that this agglutination was dependent on the presence of specific antibodies; but since any one organism, in addition to removing completely the specific agglutinin acting on itself, might reduce the titre of the serum for other organisms, and a similar general reduction in titre might be produced by adsorption with some non-specific agent such as charcoal, he concluded that another non-specific serum constituent was concerned in the full effect obtained with the untreated sera. In later experiments (Gibson 1932), in which the H and O

antigens and antibodies were differentiated, he obtained results suggesting that the specificity was due mainly to the H agglutinins, while the O agglutinins for any one bacterium might be greatly reduced by absorption with an antigenically unrelated organism.

Timmerman (1930) tested sera from the guinea-pig, rabbit, hare, goat, sheep, buffalo and man against *Salm. typhi* and was able to demonstrate the presence of both somatic (O) and flagellar (H) agglutinins in one or more specimens of serum from each species, except in the case of the guinea-pig, in which 2 of 20 animals showed O but no H agglutinins.

Lovell (1932) tested the sera of 202 normal swine, 23 cattle, 46 sheep and 20 horses for flagellar agglutinins against 7 different types of salmonella bacilli. Normal agglutinins against one or more of these types were present in 190 swine, 13 cattle, 36 sheep and 18 horses, usually to relatively low titre. Absorption experiments indicated that these agglutinins were in most cases specific. In further studies (Lovell 1934) he tested the sera of 263 normal swine, 163 cattle, 71 sheep, 40 horses and 40 rabbits for H and O agglutinins against *Salm. typhi-murium*, *Salm. newport*, *Salm. choleræ-suis* and *Salm. enteritidis*. The great majority of the pig, cattle, sheep and horse sera showed the presence of flagellar agglutinins for some, or all, of these species. A rather smaller, and more variable, proportion showed the presence of somatic agglutinins. As in the earlier study, absorption tests gave clear indication of specificity, though in some instances there were minor degrees of cross-absorption. The rabbit sera seldom showed the presence either of flagellar or somatic agglutinins. Mackie and Finkelstein (1930, 1931, 1932) (see also Finkelstein 1933) have tested the serum of a number of normal animals, such as the ox, sheep, horse, pig, rabbit, rat, cat, guinea-pig, pigeon and man, against a variety of bacteria, including *V. choleræ*, *Salm. typhi* and other members of the *Salmonella* group, dysentery bacilli, proteus bacilli, influenza bacilli, *Br. abortus*, *Br. melitensis*, meningococci and so on. They have also (Mackie *et al.* 1932) tested the bactericidal action of whole blood and of serum-leucocyte mixtures. They were able to demonstrate complement fixation and bactericidal action in a large number of cases, and to show that the bactericidal effect depended on the joint action of complement and a heat-stable factor that could be removed by absorption. This absorption was specific, in the sense that it was often possible to demonstrate the removal of bactericidal power against the absorbing organism while the absorbed serum was still bactericidal for other bacteria.

Gordon and Carter (1932) record results which lead them to a different conclusion. Testing rabbit and guinea-pig sera against cholera vibrios, typhoid and other salmonella bacilli, and dysentery bacilli, they were able to demonstrate a well-marked bactericidal action that depended on the interaction of complement and some heat-stable factor; but they failed to demonstrate any specific absorption of the latter. Their results suggested that the bactericidal activity against the less sensitive organisms could be removed by absorption with many different bacteria, leaving in each case an effective residuum of bactericidal action against those organisms, such as the cholera vibrio and the dysentery bacillus, that are particularly sensitive to serum lysis. Gordon and Johnstone (1940) later demonstrated a certain degree of specificity in normal rabbit, guinea-pig and human sera, in that, though absorption by heavy suspensions of one bacterium reduced the bactericidal power for a number of unrelated organisms, there was a much greater reduction of bactericidal power for the absorbing bacterium. They suggested as a possible alternative to the hypothesis of strain-specific antibodies, a specific alteration of a general bactericidal antibody during the process of exposure to the "absorbing" organism.

To summarize these findings: the serum of most normal animals contains substances that react with bacteria in the test-tube giving agglutination, complement fixation and bacteriolysis (bactericidal action). The sera of some animals, such as pigs and cattle, seem to react with a wider variety of bacteria than do the sera of other animals, such as the rabbit and guinea-pig. Different species of bacteria also vary in their sensitiveness to normal sera. Some species are agglutinated, or lysed, by normal serum from many different animals, others are sensitive

only to a few. The specificity of the flagellar agglutinins in normal sera seems to have been clearly established, and the evidence is definitely in favour of the specificity of some, at least, of the agglutinins acting on the antigens at the surface of the bacterial cells. These agglutinins, flagellar and somatic, are, it should be noted, usually present in low titre only (1 : 5–1 : 40 or thereabouts). The evidence with regard to the specificity of the bactericidal antibodies is more conflicting; but results indicating specificity have been recorded, and if somatic agglutinins are present we should expect them to exert a bactericidal action in the presence of complement.

In view of the divergence of evidence and opinion referred to above, it may be well to discuss this question of specificity a little more fully. The conclusion that the natural antibacterial sensitizers are specific does not, of course, imply any particular hypothesis as to their mode of origin; it assumes only that they unite with certain bacteria because of a chemical affinity conferred by certain specific combining groups.

We need not, therefore, assume that the natural antibodies are in all cases identical with those induced by active immunization. A "natural" antibody and an "immune" antibody, both acting on the same bacterium, must each possess chemical groupings with a specific affinity for groupings borne by the bacterium; but, if the natural antibody has arisen in some way other than in response to infection with the actual bacterium concerned, it is quite possible that the chemical correspondence between its active groupings and those on the bacterial surface will be less close than in the case of an antibody produced in response to infection or artificial immunization. There are certain experimental findings that are in accord with such a possibility. It is, for instance, a very general experience that some, at least, of the "natural" antibodies require, for their removal from a serum, an absorbing dose of bacteria very much larger than that required for the removal of the same amount of an "immune" antibody.

If we adopted this view we should expect that the antibacterial antibodies demonstrable in any random sample of a human or animal population would be of two kinds, those arising as the result of infection with the bacterium on which they act, and those arising in some other way. This brings us directly to the question of their origin under natural conditions.

Some Examples of Natural Agglutinins.

It is, of course, well established that certain kinds of "natural antibodies" are produced in the absence of any environmental stimulus, and in accordance with definite genetic laws.

The best example is perhaps provided by the isohæmagglutinins that divide mankind into different blood-groups.

Landsteiner (1901) distinguished the principal blood-groups into which the human race may be divided, and showed that this grouping depends, in the main, on the distribution of two different antigenic constituents, which may be present together, or separately, or may both be absent. There are two corresponding antibodies, and these are so distributed that neither antibody is present in the blood of an individual whose red cells contain the corresponding antigen. By determining the antigens present in any sample of blood it is therefore possible to assign it to its correct group. Two ways of numbering the four main blood groups were devised (Jansky 1908, Moss 1910), which in the past led to confusion. The terminology of the groups adapted from that of von Dungern and Hirzfeld (1910–11) is to be preferred. The antigens are referred to by a

capital letter, the antibodies by Greek letters, and the blood group to which an individual belongs is conveniently named according to the presence or absence of the A and B antigens. The constituent factors of the four groups can then be set out as follows:

Antigens	O	A	B	AB
Antibodies	$\alpha\beta$	β	α	—

The serum of a Group O blood will agglutinate the corpuscles of Groups A, B and AB, for it contains the antibodies α and β; but the corpuscles of this group will not be agglutinated by the serum of any group, since they contain neither of the antigens A and B. A person belonging to this group is a "universal donor"; his blood can be transferred with safety to any recipient, but he can only receive with safety the blood of a person belonging to the same group as himself. The interactions of the other groups will be obvious, and it will be noted that a member of Group A can receive blood from Groups O and A, a member of Group B from Groups O and B, while a member of Group AB can receive blood from any group, since his serum contains no antibodies, but can donate blood only to a member of his own group. It should be noted that there is an antigen characterizing Group O cells, but the occurrence in human sera of antibodies specific for O is extremely rare. The blood cells of man are also characterized by one, or both, of two antigens M and N (Landsteiner and Levine 1928) but, as with the O antigen, naturally occurring antibodies to M and N are exceedingly rare. Recently Landsteiner and Wiener (1940, 1941) discovered that the sera of rabbits immunized by the erythrocytes of the *rhesus* monkey would agglutinate those of certain human subjects, and added the Rh antigens to the list of antigens already characterizing the human erythrocyte. The presence or absence of these antigenic components, and of the various subgroups that have been described, is determined genetically according to Mendelian principles; the A, B and O antigens form one system, in which A and B are dominant to O (Hirzfeld 1926, Bernstein 1924); the M and N form a second (Landsteiner and Levine 1928) and the Rh antigens a third, in which the presence of Rh is dominant to its absence (Wiener 1942, Race and Taylor 1943, Race, Taylor *et al.* 1943, Race 1944).

This brief description does not include some minor antigens present in certain blood cells and must necessarily ignore the far-reaching developments in genetics, in the pathology of blood diseases and in immunochemistry, that have arisen from Landsteiner's original discovery. We are concerned here with the relation of certain antigens to the presence of antibodies in the blood. The presence of two antibodies, the α and β agglutinins, is either genetically determined, or is dependent on some other genetically determined factor such as absence of the corresponding antigens in the red cells of the blood.

The Rh antigen is of particular interest. Unlike the A and B, and like the O, M and N antigens, its presence in the cell is not invariably associated with the absence in the serum of corresponding antibodies. In certain circumstances, in fact, it is antigenic in man; and the conditions in which it is able to immunize man, and the consequences of that immunity, are highly instructive from the immunological point of view. Among British and white American people, about 85 per cent. of the population are Rh-positive, the remainder Rh-negative. If an Rh-negative woman mates with an Rh-positive man, the fœtal erythrocytes may be Rh-positive, and in a certain number of cases, depending perhaps on the condition of the placenta, the Rh antigens of the fœtus are transferred to the mother, and induce the formation of Rh-agglutinins. Towards the end of pregnancy, these agglutinins appear in the circulation of the fœtus, and, uniting with the fœtal erythrocytes, sensitize them to lysis, in a manner analogous with the sensitization, let us say, of Group A cells transfused into a Group B person with natural α agglutinins in his serum, The resulting destruction of the red cells may lead to the death of the fœtus and abortion, or the birth of an infant with the syndrome known as erythroblastosis fœtalis (Levine. Burnham, Katzin and Vogel 1941, Levine, Katzin and Burnham 1941, Boorman, Dodd and Mollison 1942). We have here a clear-cut example of the absorption of antigens through the uterus, and of the placental transmission of maternal antibodies. The Rh

antibody may also be transmitted by the colostrum. There is clearly a difference between the action of maternal Rh-agglutinins and the maternal α and β agglutinins, for it is only rarely that marked blood destruction occurs in infants of the A, B, or AB groups whose mother's blood contains corresponding natural agglutinins. We may postulate a selective transmission of Rh, but not α or β agglutinins, or that the infants are in some way insusceptible to α and β agglutinins. The second explanation seems more likely to be correct. It has been suggested that the cells of the infant are protected from α and β agglutinins of the mother by water-soluble Group A or B substances which are present in the tissues and body fluids of the infant, and which unite with any maternal agglutinins that reach the fœtus. It is known that in the majority of persons, the group A and B substances of the red cell are also present in the tissue fluids; they have been found, for example, in urine (Yosida 1928), gastric juice (Witebsky and Klendshoj 1940), saliva (Landsteiner and Harte 1941) and in the secretions of pseudomucinous ovarian cysts (Morgan and van Heyningen 1944). The Rh substance, on the other hand, is confined largely to the erythrocytes (Levine and Katzin 1941, Wiener and Forer 1941) and to the fœtal tissue cells (Boorman and Dodd 1943), but is not present in the tissue fluids, and, as a result, is not available for the neutralization of maternal Rh-agglutinins. (For a full discussion, see Levine 1943). The effect of A and B substances is analogous in one respect with that postulated in Chapter 48 for the aggressive action of bacteria; in the one case the soluble bacterial antigens neutralize antibacterial antibody, and so protect the bacterial cell from opsonization, and in the other, soluble erythrocyte antigens protect the red cell from specific sensitization to lysis

In addition to these isoagglutinins and isolysins—antibodies acting on the corpuscles of other individuals within the same species—the sera of all animal species that have been examined have been found to contain antibodies acting on the red cells of certain other animal species. The kind, number and activity of these antibodies vary from one species to another in the same way as the bacterial sensitizers described earlier in this chapter. The pioneer researches of Ehrlich and Morgenroth (1899, 1900*a*, *b*) established quite clearly, by means of absorption tests, the specificity of the antibodies concerned.

Forssman and Related Antibodies.

In this connection and because it has a direct bearing on the problem with which we are mainly concerned—the origin and nature of the natural antibacterial sensitizers—brief reference must be made to an antigen-antibody reaction of a curious and interesting kind which was first described by Forssman (1911), who observed that the injection into rabbits of organs obtained from the guinea-pig, or from certain other animals, led to the production of hæmolysin for sheep's red corpuscles. These observations have been confirmed and extended by many subsequent workers (see Taniguchi 1919–20, 1921, 1922, Browning 1931); and it is now known that the so-called *heterophile* or *Forssman* antigen, which is responsible for this reaction, is widely distributed among different animal species, and in a curiously random fashion. Thus the organs of the guinea-pig, horse, dog, cat, mouse, fowl and tortoise stimulate the production of heterophile antibody when injected into the rabbit, while the organs of man, rabbit, ox, sheep, rat, goose, pigeon, eel and frog do not. The organs and tissues that have been examined with regard to the presence or absence of heterophile antigen include kidney, liver, lung, adrenal, testicle, heart, skeletal muscles, lymph glands and serum, though not all of these have been tested in the case of each of the species enumerated above. It is noteworthy that the organs of the sheep, freed of all red corpuscles, do not stimulate the production of heterophile antibody; and in general it appears that those species that contain heterophile antigen in their red corpuscles do not contain it in their tissues, at least in the full and effective antigenic form.

It soon became clear, from absorption experiments of the usual type, that the hæmolysin produced by the rabbit in response to the injection of guinea-pig's kidney was not

identical with that produced in response to injection with sheep's corpuscles. The hæmolytic serum in the latter case contains a mixture of hæmolysins, only one of which corresponds to the heterophile antigen.

It should be noted that only animals of the rabbit type—those not containing heterophile antigen in their tissues—are capable of producing heterophile antibody in response to the injection of guinea-pig's kidney or other suitable tissue. It is also of particular interest from our present point of view that certain normal antibodies are of the heterophile type—for instance, the natural hæmolysin for sheep's red cells that is found in the serum of rabbit and man (Friedemann 1917). In the case of human serum, this reaction may depend on the α isoagglutinin, for, as Schiff and Adelsberger (1924*a*, *b*) showed, there is a close antigenic relation between the heterophile antigen and the Group A substance.

The constitution of the heterophile antigen appears to vary with the organism in which it is found. According to earlier workers (Iwai 1917, Georgi 1919, Taniguchi 1921) the specific component (hapten) is associated with lipoid constituents of the tissues, especially the alcohol-soluble " lecithin " fraction. The heterophile antibody, in addition to acting as a hæmolysin for sheep cells, forms a precipitate and fixes complement when mixed with an emulsion of these lecithin fractions of tissues containing heterophile antigen. The lipoid extracts are not antigenic, but can be made so by mixing with a suitable protein, the resulting antisera containing both heterophile antibodies and antibodies specific for the protein used (Landsteiner and Simms 1923). These haptens were apparently lipopolysaccharide in nature (Landsteiner and Levine 1925, 1927 ; but see also Renaux and Thomas 1942).

The human Group A substance, which in pure form is a non-antigenic hapten, has proved to be a viscous substance made up of polysaccharide units, partly hexose, partly N-acetylglucosamine, and a number of amino-acids (Goebel 1938, Landsteiner and Harte 1940, 1941, Morgan and King 1943, King and Morgan 1944). As we have indicated in Chapter 8, a number of bacteria contain the Forssman antigen or substances immunologically related to it. In *Sh. shigæ*, the Forssman hapten is associated with the lipopolysaccharide moiety of the somatic antigen (Meyer and Morgan 1935, Meyer 1938, Morgan and Partridge 1940). As Morgan showed, the hapten from *Sh. shigæ* is not antigenic, but can be made so by reunion with the protein moiety of the antigen (see p. 261). The Group A substance can in the same way be made powerfully antigenic by union with this *Sh. shigæ* protein ; and the resulting antigen, like the complete antigen of *Sh. shigæ*, stimulates the production of heterophile antibodies. This interesting demonstration, together with that of Landsteiner and Simms (1923), suggests a means whereby heterophile antigenic properties might be acquired by certain organisms, and suggests the underlying mechanism of a phenomenon observed by Holtman (1939), who induced the formation of heterophile antigens in *Salm. typhi* and *Salm. paratyphi B*, by prolonged subcultivation on media containing Forssman antigen (horse serum) or by growth in collodion sacs implanted in the peritoneal cavity of guinea-pigs. This phenomenon makes it clear, at all events, that the cultural history of bacterial antigens employed may be of great importance in the investigation of heterophile antigens ; and the demonstration that bacteria acquire Forssman antigens by contact with animal tissue, as Holtman points out, may have some bearing on the fact that the bacteria which are known to contain Forssman antigens are for the most part parasitic and may as a species have acquired the antigen by contact with the host's tissues.

The Forssman antigen of the pneumococcus is also related to a somatic antigen, but not, as in *Sh. shigæ*, to the dominant antigen of the virulent organism. The more recent investigations of Goebel and his colleagues (Goebel and Adams 1943, Goebel, Shedlovsky *et al.* 1943) indicate that it is a lipopolysaccharide, containing a high molecular weight fatty acid, an acetylated amino sugar and a second hexose. The polysaccharide is closely related to, if not identical with, the somatic " C " polysaccharide of the pneumococcus described by Tillett, Goebel and Avery (1930). It is noteworthy that an antibody to this C substance appears in the serum of patients in the acute febrile stage of infection

with pneumococci and a number of other bacteria (Tillett and Francis 1930, Ash 1933, Francis and Abernethy 1934, Abernethy and Francis 1937). The same kind of antibody can be induced in *Macacus* monkeys by infection with pneumococci, but not in rabbits or mice (Abernethy 1937). The antibody or "C-reactive protein," is unusual in many respects. It appears rapidly in the serum of the infected animal and develops with the inflammatory reaction, whether pneumococcal or otherwise; it persists only during the activity of the lesion, and in pneumonia disappears before the crisis; it precipitates with the C polysaccharide only in the presence of calcium ions, and is precipitated from serum by salts along with the albumin fraction (Abernethy and Avery 1941); it is antigenic, and antigenically distinct from the normal proteins of human or monkey sera, though the C-reactive proteins of man and monkey are antigenically similar (MacLeod and Avery 1941); and in the electrophoretic tube it migrates neither with albumin nor the β- and γ-globulins, but is an apparently new type of protein migrating with the α-globulins (Perlman, Bullowa and Goodkind 1943). This antibody, the "C-reactive" protein, is similar to one found by Löfström (1939, 1942, 1943, 1944), in human sera, which agglutinated with, and induced a capsular swelling reaction in, certain pneumococcal types. Its relation to heterophile antibodies is not clear. Lofström found that it was formed in the acute stages of non-infectious disease in which there was marked tissue destruction, and was able to induce its appearance by the injection, for example, of colloidal sulphur.

In the C-reactive protein we have therefore an example of an antibody-like substance which at first sight appeared to be specifically induced by an antigenic component of a bacterium, with which it reacted in an apparently orthodox manner, but whose production was found on analysis to be conditioned by the species of animal, and by a number of non-specific stimuli, including bacterial infections. It at least provides a firm factual basis for a hypothesis of a natural antibody formation which is independent of stimuli by the kind of antigens we have discussed in Chapter 7, and which may be independent of the mechanism of antibody-formation discussed in Chapter 50.

We may note that heterophile antigens and antibodies other than those of the Forssman type occur in animal tissues. Thus Deicher (1926) records that the injection of horse serum into man induces the formation of agglutinins not only for sheep erythrocytes, but also for those of the rabbit, which contain no Forssman antigen. The distinction of these agglutinins from Forssman antibody was confirmed by Schiff (1937) who demonstrated them in man following injection of rabbit serum, which also contains no Forssman antigen. There appeared to be two antigens concerned, one specific for rabbit erythrocytes, the other shared with those of the sheep, horse and ox. Graña (1944) records similar antibodies in patients with hydatid cysts, who have been injected with hydatid fluid. Brown (1943) found an additional thermolabile antigen in mouse tissues distinct from, but related to, the Forssman antigen. (For a discussion of an apparently heterophile antibody of yet another type, which characterizes the serum of patients with infectious mononucleosis, see Bernstein 1940).

Age and Natural Antibody Production.

We may note that normal hæmolysins and hæmagglutinins are absent from the blood of very young animals, and are developed during the process of normal growth (Hirszfeld 1926, Friedberger, Bock and Fürstenheim 1929). The same is apparently true of the natural antibacterial agglutinins (Kraus and Low 1899, Lüdke 1905, Braun 1909, Gibson 1930; see also Mackie and Finkelstein 1928, 1930). Both Rywosch (1907) and Sherman (1919) noted that the normal hæmagglutinins of the fowl did not appear in the chick embryo until the twenty-first day, though Rywosch observed lysins of *Bact. coli* as early as the fourteenth day. In this connection it may be noted that Polk, Buddingh and Goodpasture (1938) could find no complement in the chick embryo. It appeared suddenly in the newly hatched chicken, and gradually increased with age. Observations made by Bailey (1923) add a point of considerable significance. He also noted the absence of normal hæmagglutinins in the young chick, and their appearance during the normal

process of growth, but he also found that chickens failed to respond to immunization with foreign red cells until they had spontaneously developed some agglutinating capacity. The inability of the antibody-forming apparatus to respond effectively to an antigenic stimulus during the earliest period of life would seem to be a general rule. Thus, Blum (1932) and others have noted the failure of young infants to produce diphtheria antitoxin in response to the injection of toxoid. The antibody response of young rabbits to the injection of bacteria, erythrocytes, or of soluble protein antigens is poor (Freund 1930). Baumgartner (1934) found that production of agglutinins to *Salm. enteritidis* given intravenously to rabbits was not maximal until maturity was reached. The response in 50-day-old rabbits was poor, and moderate at 70 days. It is also noteworthy that antibody from the younger animals had a lesser "avidity" than that from the mature rabbit. Baumgartner (1937) later observed a similar age difference in the response to ovalbumin, and showed that the combining power of the precipitins produced increased with the age of the immunized animal.

In Chapter 7 we have given reasons for believing that antibodies are specialized serum globulins. It is therefore of particular interest to find that the serum of new-born animals is markedly deficient in globulin, particularly in euglobulin, that this deficiency is temporarily made good in certain species by the transfer of globulin from mother to young in the colostrum, and that the capacity for producing euglobulin from its own tissues is developed by the young animal at a later stage of growth (Toyama 1919, Howe 1922, Lewis and Wells 1922). Both Jameson, Alvarez-Tostado and Sortor (1942) and Polson (1943) record striking changes in the serum proteins of calves and horses after birth, as determined by electrophoretic analysis. Polson records an increase in protein concentration from 3·7 per cent. at birth, to 5·25 per cent. after 5 days' suckling. At 8 months the concentration was 6·8 per cent. At birth the predominant protein was albumin (65 per cent.) and the remainder mainly α-globulin. The β- and γ-globulins, with which, it will be remembered (Chapter 7), antibodies are associated, were absent, or present only in small quantities at birth, increased rapidly in the nursing period, and at 8 months together constituted 50 per cent. of the serum proteins. The appearance of the natural antibodies and the increase in serum globulin are closely correlated with one another (see Famulener 1912). Orcutt and Howe (1922) have observed the passage of natural agglutinins for *Br. abortus* from a cow to her calf in the globulins of the colostrum; and Timmerman (1931) has demonstrated the presence of agglutinins for the typhoid bacillus in human colostrum during the first 5 days of suckling.

Little is known of any association between genetic constitution and natural antibacterial antibodies. Irwin and Bell (1935) bred two lines of rabbits, one of high, the other of low resistance to *Br. suis*, and found that resistance was correlated with the bactericidal power of whole blood for the organism. The lethal power of the blood depended on the presence of bacteriotropins, which were specific for *Br. suis* in that they had little effect on *Br. abortus* or *Salm. enteritidis*; but the evidence does not permit us to decide whether it was the production of bacteriotropins, or a readier response to latent immunization by antigens identical with or similar to those of *Br. suis*, that was genetically determined.

The Origin of the Natural Antibacterial Antibodies.

It is clear, therefore, that during the normal course of its development, any animal will produce a number of natural "antibodies" differentiated from one another in possessing different specific combining groups. Some, like the α and β agglutinins in human sera, are formed as the result of an internal physiological disposition of the animal, and others in response to an external environmental stimulus, which we may or may not be able to attribute to a known antigenic substance.

The acceptance of the view that some of the natural antibodies that act on

bacteria may arise during the normal production of serum proteins, simply because of an accidental chemical affinity between certain of these proteins and certain bacterial antigens, does not involve the assumption that this is the usual method of production. It is much more likely that environmental stimuli play an important part. The most obvious of such stimuli is that provided by natural infection with the bacteria on which the natural antibodies are found to act. How does this conception of their origin accord with the available evidence ?

If we turn back to the observations of Bürgi, of Gibson, and of Lovell on normal agglutinins we at once meet with a difficulty. Can we really believe that such animals as the ox and the pig are infected during their relatively short lives with such a variety of organisms as *Proteus X*19, *Salm. typhi*, *Salm. paratyphi A*, *Salm. paratyphi B*, *Salm. enteritidis*, *Sh. flexneri*, *Sh. shigæ*, *V. choleræ*, and, doubtless, a host of other pathogenic organisms that are absent from the list only because they have never been tested ? We need not indeed postulate manifest infections. It is sufficient at some time or another for the animal to have carried the organism, presumably in the alimentary tract. Even so, so wide a bacterial experience seems unlikely, simply because we are not used to recognizing the carrier state for such a multiplicity of pathogenic species. It is, however, possible that oxen and pigs are in fact carriers of these organisms but that our cultural techniques are inadequate for their detection. Von Bunza (1937), for example, found *Salm. suipestifer* in none of 149 healthy pigs whose fæces he tested by orthodox methods of cultivation. Nevertheless, by a special technique, he was able to demonstrate its presence in 34 of the pigs.

But even if by the most fruitful methods of cultivation we were still unable to demonstrate the presence of bacteria corresponding to these antibodies, there is still the possibility that the stimulus was provided by the antigens of dead bacteria, or by materials containing a similar antigen. In defining an antigen we excluded administration by the digestive tract when demanding that it should give rise to the production of antibodies on injection into a suitable animal, because the antigen might be chemically altered while in the digestive tract, and we should in any case have no assurance that it would pass from the intestine into the tissues in an active form. But recent studies on oral immunization have made it clear that the antigenic components of dead bacterial cells do, in some cases at least, pass from the intestines to the tissues and stimulate antibody formation. It is at least possible that among the unlimited variety of other antigenic materials which gain access to the intestinal tract, some, under favourable conditions, may behave in the same way. Were this the case, there would be little cause for surprise if some few of the multiplicity of reactive groupings produced by an antibody-forming apparatus subjected to an infinite succession of widely varied stimuli should happen to show affinities to the antigenic groupings of certain species of bacteria. Nor need we limit the possibilities to the intestinal tract. There is good evidence that inhalation of antigenic material may result in an effective antibody response ; and under suitable conditions similar material may gain access to the tissues through the skin.

In a final analysis, antigenic specificity depends on chemical structure, and as we have seen in Chapter 8, widely different materials may have antigenic determinants in common. The fact that we discover natural antibodies by the use of a suspension of a bacterium in which we are interested does not necessarily indicate that they were formed in response to that bacterium. We know some-

thing of the detailed antigenic structure of certain groups of closely related bacteria but little with regard to the possible sharing of antigenic groups by biologically disparate species of living organisms. We must, then, accept the possibility that the antibodies which we detect in the blood of a high proportion of animals of a particular species, and which act specifically on bacteria A, B and C, may have been produced in response to infection with some other organisms, X, Y and Z. Lovell (1934) explored this possibility in the case of the natural agglutinins of young swine, and found in two instances that coliform bacilli isolated from the mesenteric glands induced, on injection into rabbits, the formation of agglutinins acting on certain bacilli of the *Salmonella* group. Later work has shown that the salmonella antigens are not uncommon among the coliform bacilli (see Chapter 30).

Ingalls (1937) records another instructive example. She found that the sera of normal adult rabbits contained agglutinins for a number of organisms present in the intestines and for other bacteria, including the pathogenic species, *Sh. shigæ*. The *Sh. shigæ* agglutinins were found to be identical with those for a normal inhabitant of the rabbit's intestines, an enterococcus, which apparently had a hapten in common with *Sh. shigæ*.

We have noted that antibodies of this type often show certain peculiarities. They tend to be present in low titre, and they tend to be very difficult to remove by absorption with the bacteria on which they act. So far as these particular antibodies are concerned the evidence appears to us to be definitely against the view that they are produced in response to natural infection.

This conclusion does not, however, apply to natural antibodies in general. Though the greater part of the evidence bearing on this problem has been obtained by studies of the kind outlined above, some of it has emerged from surveys of the normal antibodies present in the sera of human groups, living under different environmental conditions. Thus Silverthorne (1936) determined the nasopharyngeal carriage of meningococci in a group of human subjects, and found a high degree of correlation between the bactericidal capacity of the blood for meningococci, and their presence in the nasopharynx. It has sometimes been possible to correlate the results of such surveys with the associated epidemiological records, as in the studies on the distribution of diphtheria, or scarlatinal antitoxin referred to above (see, however, Peters 1940); and it has been shown quite clearly that the frequency of natural agglutinins for a particular bacterium, among a random sample of the human population living in a particular locality, is closely associated with the frequency of infections due to that bacterium in that locality. Examples are provided in the epidemiology of enteric infection (Chapter 69), of undulant fever (Chapter 75), and of certain virus diseases (Chapter 87 and 88).

A few surveys of a similar kind have been made in laboratory animals. Thus Bailey (1927) examined 81 rabbits taken from a laboratory stock among which cases of snuffles were occurring, due to infection with *Pasteurella lepiseptica*. Three rabbits were suffering from acute snuffles; cultures from the nose gave a copious growth of *Past. lepiseptica*, and agglutinins and complement-fixing antibodies were demonstrated in the blood stream. Nine had chronic snuffles; all of these showed the presence of *Past. lepiseptica* in the nares and agglutinins and complement-fixing antibodies in the serum. Three had suppurative lesions from which *Past. lepiseptica* was isolated; agglutinins and complement-fixing antibodies were demonstrated in their serum. Six showed no clinical symptoms of infection, but *Past. lepiseptica* was isolated from the nares; in all six agglutinins and complement-fixing

antibodies were found. Ten showed no symptom of infection and cultures from the nares yielded no *Past. lepiseptica*; in none of these could agglutinins or complement-fixing antibodies be demonstrated. Six rabbits were then taken, one of which yielded a scanty growth of *Past. lepiseptica* and showed the presence of complement-fixing antibodies to low titre, while the other five showed no evidence of infection. All six were infected with *Past. lepiseptica* by nasal instillation. All six became carriers without any symptoms of snuffles. The rabbit which was already infected showed a rise in titre of complement-fixing antibodies, and these antibodies appeared in each of the other five rabbits during the course of the succeeding three months.

There is then no doubt that the members of a herd, or population, submitted to a constant or recurrent risk of infection with a particular bacterium will tend to form antibodies acting on that particular organism in response to the stimuli provided by infection of varying severity; and the natural working of this mechanism will certainly play a large part in determining the frequency distribution of the antibodies concerned.

We must then accept the view that the naturally occurring antibacterial sensitizers, and perhaps also the natural antitoxins, may owe their origin to any one of four mechanisms: actual infection with the corresponding bacterium; infection with some other organism that shares a common antigenic component; the entrance to the tissues *via* the intestinal tract, or possibly by other routes, of non-living antigenic material capable of stimulating the production of an antibody with the active group in question; or the formation of such antibodies as a by-product in the normal functioning of the antibody-forming apparatus altogether apart from any specific external stimulus.

The Immunological Significance of the Natural Antibacterial Antibodies.

Do naturally occurring antibodies of the type considered above confer a relative immunity to the risk of natural infection, analogous to that conferred by the natural antitoxins? The evidence available does not allow of any but the most tentative answer. So far as it goes it suggests that there is sometimes a relation between the presence in the blood of a particular antibacterial sensitizer and a relatively high resistance to the corresponding bacterium; but that this relation is not a general one, and that many antibodies of this type are quite without effect on the resistance of the animal possessing them. This is not surprising. We have given reasons for believing that the natural antibacterial sensitizers acting on a single bacterium—even on the surfaces of a single type of bacterial cell—may be of several different kinds, and it seems not unlikely that some of these may have a protective value while others have not.

Kyes (1916) showed that, when virulent pneumococci were inoculated into the naturally immune pigeon, the organisms were rapidly withdrawn from the blood stream and phagocytosed by macrophages in the liver and spleen. Heist, Solis-Cohen and Solis-Cohen (1918) found that the whole blood of the pigeon was actively bactericidal for the pneumococcus while the whole blood of the susceptible mouse or rabbit was not. The serum of the pigeon was not bactericidal alone, indicating that the killing power depended on the leucocytes as well as on the constituents of the serum. That serum constituents are in fact concerned, and that among these constituents are specific antibodies, would seem to follow from the observations of Bull and McKee (1921), who studied the passive transfer of immunity from the normally resistant chicken to the normally susceptible mouse. They found that chicken serum would protect mice against virulent pneumococci of Types I, II and III; and that absorption of the chicken serum with Type I pneumococci removed

its protective power against Type I but not against Type II. Similarly absorption with Type II removed the protective power against this type, leaving the protective power against Type I.

Robertson and Sia (1924) found a marked inhibition of the growth of pneumococci in serum-leucocyte mixtures from the resistant cat or dog, but no such inhibition in similar mixtures from the susceptible rabbit or guinea-pig. More recently they have reported experiments which suggest that this bactericidal action is due to sensitization followed by phagocytosis (Robertson and Sia 1927). They sensitized pneumococci by prolonged contact with large amounts of the serum under test, and then determined the degree of phagocytosis by the leucocytes of various resistant and susceptible animals. They found that sensitization with serum from the resistant cat, sheep, pig and horse rendered the pneumococci susceptible to phagocytosis by the leucocytes of these animals, or by those of the susceptible rabbit or guinea-pig; while treatment with serum from such susceptible species as the rabbit, guinea-pig or man did not sensitize the pneumococci for the leucocytes of these animals, or for those from the resistant cat, sheep, pig or dog.

The actual number of sera from each species examined by Robertson and Sia was not large, and it would seem that their original conclusions require some modification, at least in regard to man. Thus, Robertson and Cornwell (1930), using serum-leucocyte mixtures, studied the presence of antibodies for various types of pneumococci in the serum of normal persons. All the sera examined showed the presence of antibodies for one or more of the serological types employed, but any given serum might be inactive against some types though active against others. It would seem, moreover, that the presence of antibodies against a particular type does not afford complete protection against infection with that type, since Robertson and others (1930) found that the serum of patients in the first 48 hours of an attack of lobar pneumonia often contained sensitizing antibodies for the infecting type of pneumococcus. Abdoosh (1936) studied the bactericidal power of the serum against gonococci in man and a large number of animal species. It was low in normal men and mice, poor in the guinea-pig. It was good in horses, oxen, sheep, pigs, goats, cats, monkeys, rabbits and rats, animals naturally insusceptible to the gonococcus. It is of interest to note that a similar degree of bactericidal activity was reached in man only 14 or more days after the onset of a gonococcal infection.

Some of the findings recorded in earlier sections with regard to the frequency distribution of various normal agglutinins make it very difficult to believe that they have any protective value. Thus somatic agglutinins for various organisms of the *Salmonella* group are frequently present in the blood of swine, cattle and sheep; but these species are relatively susceptible to infection with certain of these organisms (Lovell 1934). A particularly striking example of this kind of anomaly is afforded by the observation that the great majority of normal horse sera agglutinate the glanders bacillus to high titre, though glanders is bacteriologically a disease of equines (see Wilson 1934).

SUMMARY

(1) Antibodies of various kinds—antitoxins, agglutinins, bactericidal antibodies and so on—are frequently found in the serum of normal men and animals. The origin of these naturally occurring antibodies is still a matter in dispute; but it seems possible that they may arise in several different ways.

(2) The new-born animal, when born of a naturally immune mother, is endowed with a congenital passive immunity, due to transference of specific antibodies from the maternal blood through the placenta, or by way of the colostrum during

the early days of suckling. This, like other types of passive immunity, is of relatively short duration.

(3) Young animals from whose blood these congenitally acquired antibodies have disappeared and young animals in whom they are not present at birth often develop them with advancing years.

(4) There are reasons for believing that this production of antibodies is dependent on the activity of an antibody-forming apparatus that is imperfectly developed at birth, but reaches functional maturity at a relatively early period of life.

(5) It is certain that this apparatus, apart from any external stimuli, will form various antibodies—such as the normal hæmagglutinins or hæmolysins—which distinguish one animal species from another. It is possible that certain of the antibodies acting on bacteria or their products may be formed in the same way, as the result of a normal serogenesis determined ultimately by genetic factors.

(6) The more commonly accepted view is that these antibodies arise as the result of the response of the antibody-forming apparatus to external environmental stimuli, and that such stimuli usually consist of overt or latent infections with the bacterium in question. This view has been upheld particularly in relation to natural antitoxic immunity against diphtheria and scarlet fever, and in this regard there can be little doubt that it is correct.

(7) In the case of specific antibacterial immunity there are greater difficulties in accepting this hypothesis in its entirety. Samples of sera collected from a given animal species may contain antibodies active against a large number of different bacteria, and it seems difficult to believe that a single animal can have been infected with so many different organisms, some of which are not known to be natural parasites of the species to which it belongs. There are, however, good reasons for believing that some at least of the agglutinins or other antibodies found in normal men or animals have been produced in response to infection, overt or latent.

(8) It is possible that other forms of external stimuli may be concerned, such as (*a*) infection with a different species of bacterium, which shares a common antigenic factor with the bacterium with which the antibody in question has been shown to act, or (*b*) the absorption from the intestinal canal or through the lungs or skin of some antigenic material containing a similar reactive chemical group.

(9) The presence of natural antitoxin in the circulating blood is usually associated with an immunity to clinical infection, at least in the case of diphtheria and scarlet fever.

(10) The relation between the presence of antibacterial sensitizers and resistance to infection with the corresponding bacteria is very uncertain. It seems probable that some of these natural antibodies exert a protective effect.

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CHAPTER 50

THE ANTIBODY-FORMING APPARATUS AND ITS REACTIONS

In the preceding chapters we have referred freely to the serum antibodies without any attempt to describe the mechanism by which they are produced. Any adequate description is, indeed, impossible. We know too little. It is, for instance, quite clear that the formation of antibodies is associated in some intimate way with the formation of the serum proteins, but we are largely ignorant as to how these proteins are produced, or where. The scanty data at our disposal with regard to the cells and tissues probably involved and the way in which they respond to specific and non-specific stimuli are, however, well worth considering; since they throw light on several problems of practical as well as theoretical importance.

The Site of Formation of Antibodies

Ehrlich's conception of antibodies was that they were shed "cell-receptors." The antigen gained effective contact with the cell by becoming anchored, through specific chemical groupings of its own, to specific chemical groupings on "side-chains" carried by the tissue cells. This theory seemed to imply, by logical extension, the view that antibodies were produced by those cells to which particular antigens, by reason of their chemical structure, were most likely to become attached —that an antitoxin, for instance, was produced by the cells that were most susceptible to the corresponding toxin. A further implication seemed to be that any cell in the body was endowed with the potentiality of forming antibodies, should antigens with suitable side-chains present themselves to it. It would be rash to say that the possibility of such a generalized distribution of function has been excluded, but the trend of opinion, especially in recent years, has quite clearly been towards a different conception, which would regard the antibody-forming apparatus as an integrated system of cells, together constituting a functional though not a structural entity. The evidence on which this view is based may be summarized as follows.

The Local Formation of Antibodies.—Experiments designed to demonstrate the local formation of antibodies, at or in the immediate neighbourhood of the site of inoculation, have given either negative or only suggestive results.

Römer (1901) recorded observations which he regarded as demonstrating the formation of anti-abrin in the rabbit's conjunctiva; and von Dungern (1902) injected crab-plasma into the anterior chamber of one eye of each of several rabbits and recorded, in one instance, the appearance of a precipitin for crab-plasma in the aqueous humour of the injected eye before its appearance in the blood serum. But these observations have never been confirmed; and, at least in the case of Römer's results, they are open to criticism on technical grounds (see Hektoen 1911). Similarly, the results recorded by Wassermann and Citron

(1905), who injected typhoid bacilli into rabbits intrapleurally and intraperitoneally, and compared the antibody titre of the local exudate and of the blood serum at various times thereafter, afford no convincing evidence of any formation of antibodies in the serous cavities. Hektoen (1911) conducted a very careful series of experiments on the immunization of dogs with rat or goat corpuscles. He was unable to obtain any evidence of antibody formation in the anterior chamber of the eye, in the pleural or peritoneal cavity, or in the connective or muscular tissue of the leg. In every case antibodies appeared in the blood, but there was no evidence that these were produced at the site of inoculation. The experiments of Walsh and Cannon (1938) suggest that antibody is formed locally in response to a direct application of antigen. The antibody content of the blood, and of organs after removal of the blood by perfusion, was measured after active immunization with salmonella bacilli. After intraperitoneal injections, agglutinins were found to be distributed evenly in the nasal mucosa, lungs and spleen, in each of which the ratio of tissue to serum titres was 1 : 10. After intranasal inoculation, the tissue-serum ratio in the nasal mucosa was lower than that in the spleen, and after simultaneous intranasal and intraperitoneal inoculations with different antigens, the splenic ratio was lower for the intraperitoneal than for the intranasal antigen and the nasal and lung ratios lower for the intranasal than for the intraperitoneal antigen.

Cannon and Sullivan (1932) record experiments which they regard as indicating the local formation of antibodies at the site of an intradermal injection; and Trawinski (1932) concludes, from his experimental findings, that the skin is involved in agglutinin production irrespective of the site of inoculation of the antigen.

Hartley (1940) injected a mixture of vaccinia virus and an aluminium hydroxide gel intradermally in rabbits. A nodule, in which the cellular reaction was predominantly macrophage, was produced at the site of inoculation. Extracts of the 4-day-old nodules contained from 2 to 10 times as much antibody as blood or other tissue extracts (see also Oerskov and Andersen 1938, DeGara and Angevine 1943). Burnet (1941), on the other hand, could find no evidence of local antibody formation in the skin of rabbits by direct injection of a variety of soluble and particulate antigens. However, in the case of the positive results the part played by the dermis may be ascribed to its richness in reticulo-endothelial cells; so that these observations are in accord with those described in the following section.

The Rôle of the Reticulo-Endothelial System in the Formation of Antibodies.

Hektoen and Carlson (1910) injected dogs with rat or goat corpuscles and bled them dry from the carotid artery during the latent period of immunization, before any rise had occurred in the antibody titre of the serum; immediately thereafter they restored their blood volume by transfusion from a normal dog. This procedure appeared to have, if anything, a stimulating effect on the subsequent antibody production, indicating that the injected antigen had been rapidly removed from the blood. This suggestion was confirmed in other experiments in which normal dogs were bled dry and transfused with blood from other dogs 3 to 48 hours after inoculation of the antigen. When the dose of red cells injected into the donor had been of the usual optimal amount the recipient showed no rise in antibody titre, indicating that no appreciable amount of antigen remained in the circulating blood even after so short an interval.

We need only recall the description of the clearing mechanism given in Chapter 47 to evoke the suggestion that the cells of the reticulo-endothelial system, which we know to be concerned in the removal of foreign particulate material from the blood stream, are also concerned directly or indirectly in the formation of antibodies.

The view that certain of the organs or tissues which we now group together under this system might be specially concerned in antibody formation is by no means new. Pfeiffer

and Marx (1898) stated that in rabbits, which were undergoing immunization against cholera vibrios, bacterial antibodies might be present in the spleen, the bone-marrow, and perhaps the lungs, in higher concentration than in the blood (see also Tsurumi and Kohda 1913, Cary 1922).

Deutsch (1899) studied the effect of splenectomy on the production of agglutinins and protective antibodies after intraperitoneal injection of typhoid bacilli into guinea-pigs, and found that splenectomy before the injection of the bacterial antigen had little or no effect on the subsequent antibody production, while splenectomy carried out 3 to 5 days after the immunizing injection led to a significant decrease in the concentration of antibodies. He added the interesting observation that, when the spleen was extirpated from a recently immunized guinea-pig and transferred to the peritoneum of a normal guinea-pig, anti-typhoid agglutinins appeared in the blood of the latter though to a relatively low titre. Luckhardt and Becht (1911) studied this question in greater detail. Dogs were injected intravenously with rat or goat corpuscles. Shortly afterwards the spleen was removed, emulsified, and injected into the peritoneum of a normal dog. The recipients showed the typical rise in the antibody titre of the blood serum. The animals from which the spleen had been removed after injection of the red cells produced hæmolysins, hæmagglutinins and hæmopsonins less rapidly and to a lower titre than did the control animals not submitted to splenectomy.

The experiments of Topley (1930) on agglutinin formation in rabbits gave analogous results, and afford the additional suggestion that, after minimal doses of antigen, the reticulo-endothelial cells do not release the antigen in an unaltered form. Rabbits received intravenous injections of killed paratyphoid bacilli in a dose of 10^5 bacilli per kilo. body weight. At varying intervals thereafter, 24 hours to 216 days, they were bled and killed. The spleen, emulsified in saline, was in each case injected into a normal rabbit. The serum from a rabbit killed 24 hours after the injection of bacilli contained no agglutinins. The rabbit that received the spleen tissue from this animal developed agglutinins to low titre, but the time relations of the response differed from those of the typical response to active immunization (see pp. 1106–1114) in several ways. The highest titre was reached earlier, and the agglutinins disappeared far more rapidly from the blood stream. Reactions of the same general type were noted in the normal rabbits injected with the spleens removed from the immunized animals after longer intervals. Moreover, these rabbits, when injected 21 to 62 days after receiving the spleen tissue with a minimal dose of the same bacilli, showed no evidence of the energetic secondary response (see pp. 1110–1114) which might have been expected if they had received a previous stimulus with unaltered antigen. These results are more compatible with the view that the histiocytes of the spleen themselves form antibodies or some intermediate product than with the view that they merely retain the antigen and afterwards liberate it in a form which provides an antigenic stimulus to other antibody-forming cells. DeGara and Angevine (1943) measured the antibody content of skin, bone marrow, liver, spleen and in some cases the lymph nodes, in rabbits inoculated intracutaneously with a single dose of streptococcal or pneumococcal vaccine. They found antibody was present in these sites before any antibody appeared in the blood, and persisted there after the blood levels had started to decline. They concluded that besides the organs naturally rich in macrophages, the inoculation site in the skin, and the local lymph nodes produced antibody, perhaps with the aid of macrophages accumulating therein as a result of the inflammatory reaction to the vaccine.

Scarff (1931) has recorded experiments suggesting that the reaction of the spleen to foreign blood corpuscles is different from its reaction to bacteria; and it may be that we should consider different antigenic materials—foreign cells, bacteria, antigens in solution and so on—in relation to different depôts of histiocytes, rather than antigens in general in relation to histiocytes in general.

The rôle of the reticulo-endothelial system in the production of antibodies has also been investigated by the method known as *blockade*, alone or combined with splenectomy. In this method large amounts of Indian ink or colloidal iron are injected intraven-

ously, with the object of engaging the activities of as large a quota as possible of the reticulo-endothelial system, and so rendering it less able to deal with a subsequent injection of antigenic material. The results recorded show some divergence. Bieling and Isaac (1921, 1922*a*, *b*) reported an inhibitory effect of blockade on antibody production, and several workers have obtained analogous results (Siegmund 1922, Gay and Clark 1924, Vanucci 1924, Jungeblut and Berlot 1926, Lewis and Loomis 1926, Meyer 1926, Tuft 1934). Others, however, have failed to detect any difference between the behaviour of normal and blockaded animals (Rosenthal and Fischer 1922, Fränkel and Grunenberg 1924, Ross 1926). Such discrepancies need cause no surprise. In the case of a system of cells so widely distributed throughout the body, the effectiveness of any method of blockade must vary according to the conditions obtaining in each particular case. Blockade that falls short of completeness may, as some authors have suggested (Standenath 1923), have a stimulating rather than a depressing effect. Philipson (1936) demonstrated the association of the reticulo-endothelial system with antibody formation in another manner. He measured the degree of blockade achieved after the intravenous injection of particulate matter by the diminution in the rate of absorption of congo red from the blood stream of rabbits, and found that whereas a primary injection of a typhoid vaccine had little blockading effect, a secondary injection 6 days later produced a blockade equal to that of large doses of Indian ink, and depressed the antibody level in the serum. One to two days later, the blockade effect diminished and at the same time there was a rise in the antibody content of the blood.

Other methods of injuring or disabling the cells of the reticulo-endothelial system have been utilized in attempts to study the part played by these cells in antibody production—the action of X-rays (Benjamin and Sluka 1908, Lawen 1909, Hektoen 1915, 1918, 1920), of thorium X (Hektoen and Corper 1920), and of radium emanation (Hektoen and Corper 1922), of benzene (Rusk 1914, Simonds and Jones 1915, Hektoen 1916), of toluene (Hektoen 1916*b*, Weaver *et al.* 1943) and of mustard gas (dichlorethyl sulphide) (Hektoen and Corper 1921). The results of these experiments, taken as a whole, lend support to the view that any procedure which severely injures the cells of the reticulo-endothelial system will markedly, or sometimes completely, inhibit the formation of antibodies. Their significance is, perhaps, rendered a little dubious by the fact that the doses required to produce a clear-cut inhibitory effect were of an order which makes it unlikely that their action was confined to the cells of the reticulo-endothelial system and the hæmopoietic tissues.

Sabin (1939) attempted to correlate the disappearance of a coloured azo-dye protein antigen from the reticulo-endothelial cells of the rabbit, with the antibody levels found in the serum. Her results suggest a connection between the two phenomena, but the antibody response in the few animals tested was too variable to permit any definite conclusions to be drawn.

The Rôle of Lymphoid Tissue in the Formation of Antibodies.

The association of lymph nodes with antibody formation was first clearly demonstrated by McMaster and Hudack (1935) who injected bacterial vaccines into the ears of mice, and after 2 hours, when some of the inoculum had passed *via* the lymphatic channels to the cervical lymph nodes, amputated the ear. In the early stages of the antibody response, the antibody content was highest in extracts of the nodes, and relatively low in the serum. The vaccine caused some inflammation in the lymph nodes, but the observed antibody levels were not due to an abnormal concentration of serum antibodies in the inflamed node, for when both ears were injected, each with a different vaccine, in each case the antibody levels attained were in the descending order—homolateral cervical node, blood serum, contralateral cervical node—though both cervical nodes were equally inflamed. McMaster and Kidd (1937) record similar results with vaccinia virus injected into the ears of rabbits, and Burnet and Lush (1938) with influenza virus given intranasally in mice, the highest antibody levels in this case being found in the mediastinal glands.

When subcutaneous injections of bacteria or bacteriophage are made into the leg of a rabbit, the early antibody response is highest in the popliteal node on the injected side (Burnet 1941). Murakami (1936) obtained higher titres in efferent lymph from nodes draining the site of injection of the antigen, than in the blood stream, but attributed the result to a concentration of serum antibodies in the inflamed node.

It is possible that the reticulo-endothelial cells of the lymph nodes are responsible for the local formation of antibody. Lymphoid tissue contains a number of "germinal centres," which are nodules of apparently primitive lymphocytes surrounded by mature lymphocytes, and are thought to be concerned in the genesis of lymphocytes. Hellman and White (1930), however, observed that the injection of antigenic material was followed by an increase, sometimes very marked, in the number of "germinal centres" of the lymphoid tissue of the spleen and the lymph nodes. Moreover, the "germinal centres" are not found in the fœtal tissues. According to Glimstedt (1936) they do not develop in the lymphoid tissue of young guinea-pigs reared in a sterile environment, but are plentiful in those that have been exposed to environments containing bacteria. The association of newly appearing centres with the stimulus of foreign antigenic material was confirmed by Österlind (1938) who found that diphtheria toxoid in the hind leg of a rabbit induced their formation maximally in the nodes draining the injected area, and that maxima of antitoxin formation, particularly in response to a secondary dose of toxoid, coincided with the maxima in numbers of germinal centres. Similar results were obtained by Ehrich and Harris (1942), using typhoid bacilli, sheep erythrocytes, and ovalbumin as antigens; in their hands the antibody maxima in efferent lymph and in the lymph nodes slightly preceded the maxima in germinal centres, whose increased numbers persisted some time after antibody levels were declining. More recently, Harris, Grimm, Mertens and Ehrich (1945) report that not only is the rise in antibody content of lymph from an immunized lymph node accompanied by a rise in the lymphocyte content, but that these cells contain substantially more antibody than the fluid itself. Moreover, since the cells can be made readily to yield antibody, but not to absorb it, it appears that the lymphocyte itself may be the actual site of antibody formation. There is, then, good, but not very extensive, evidence for the formation of antibodies in lymph nodes, and strong indications that tissues, which, though mesenchymal in origin, are not reticulo-endothelial in nature, may be concerned in antibody production. It should be noted that Burnet (1941) was unable to demonstrate any antitoxic response in lymph nodes to staphylococcal toxoid; he suggests that toxoid, being soluble, is not held up in the lymph nodes as effectively as particulate antigens are.

The Rôle of Other Organs or Tissues in the Formation of Antibodies.

It may be noted that the effect on antibody formation of the removal of other organs —unconnected with the reticulo-endothelial system—has been studied by various observers. The removal of the thyroid has apparently no inhibitory effect (Fjeldstad 1910, Hektoen and Curtis 1915, Ecker and Goldblatt 1921, Houssay and Sordelli 1921, Clevers 1921, Také 1923). An increase in antibody production has occasionally been recorded as a result of this procedure (Ecker and Goldblatt 1921, Houssay and Sordelli 1921, Clevers 1921). Hektoen and Curtis (1915) state that complete removal of the stomach or of the small intestine does not interfere with the formation of hæmopsonins or hæmagglutinins; simultaneous removal of the spleen and pancreas appears to have the same depressing effect on antibody production in dogs as removal of the spleen alone; adrenalectomy in dogs, at the height of the antibody curve, does not alter the antibody content of the blood serum; in rats, removal of one half of the liver appears to have no effect on the production of hæmolysin.

The Formation of Antibodies in Tissue Cultures.—A method which offers obvious possibilities for the study of antibody formation is that of tissue culture.

Carrel and Ingebrigsten (1912) cultivated fragments from the bone-marrow and lymph glands of guinea-pigs in the homologous blood plasma, and added to the cultures small

amounts of red cells from the goat, against which the serum of the guinea-pig showed no lytic action. Hæmopsonins appeared in the culture on the 3rd day, as judged by direct observation of the degree of phagocytosis. Hæmolysins were detectable on the 4th day, and had increased markedly in activity on the 5th. The hæmolysins could be specifically absorbed from the fluid by the usual technique. Ludke (1912) injected killed cultures of typhoid or dysentery bacilli into rabbits, removed fragments of the spleen or bone-marrow after 1 to 5 days, and cultivated them in homologous plasma. After 2 to 5 days' incubation he was able to detect lysins and agglutinins in the culture fluid. In a similar way he succeeded in producing hæmolysin for ox or sheep corpuscles. His results were for the most part negative when he added the antigenic materials directly to the tissue cultures. Przygode (1913, 1914), Reiter (1913) and Schilf (1926) have also recorded positive results. Meyer and Loewenthal (1928) record experiments in which they were able to demonstrate a significant rise in agglutinins in tissue cultures prepared from rabbits that had been inoculated with typhoid bacilli. The tissues examined were the spleen, the lymph glands and the milk spots from the omentum. The latter observation is highly significant, since the omental milk spots, in addition to fibroblasts, contain only cells of the reticulo-endothelial type. Subsequent workers have demonstrated antibody formation against a variety of antigens when the cultures were made from the tissues of already inoculated animals; and the results of adding the antigen after the establishment of the tissue culture *in vitro* have been uniformly negative (Hwon 1937, Parker 1937, Salle and McOmie 1937, Beard and Rous 1938). The reasons for the failure of *in vitro* immunization are not apparent, though Beard and Rous, who used a pure culture of Kupffer cells from the liver, note that the cultivated cells had lost their normal *in vivo* power of ingesting antigenic particles.

Conclusions with regard to the Site of Formation of Antibodies.—Taking the data as a whole the conclusions to be drawn do not seem to be in doubt in spite of minor discrepancies. There is ample evidence that the organs and cells which have been shown to be concerned in the clearing mechanism of the tissues are also concerned in some way with antibody formation. There is also evidence that lymphoid tissue may be concerned. It is clear that antibody formation is not the function of mesenchymal cells in any one organ of the body. Thus Buttle (1934), in short-term experiments with rabbits in the early stages of a secondary antibody response (p. 1110), was unable to interrupt the flow of antibodies from the tissues to the blood stream by extirpation of the liver and spleen, or by complete removal of the skin.

Of the precise part played by the cells of the reticulo-endothelial system we know little. We know that they remove from the blood stream particulate antigenic material, and probably similar material in colloidal solution. Do they themselves produce the corresponding antibodies, or do they merely retain the antigen temporarily, yielding it up in an altered or unaltered state to the tissue fluids, and thus passing it on to some other cells in which the actual antibody synthesis occurs?

Of the experiments outlined above, some of those dealing with the rôle of the spleen in antibody production, the results obtained by Meyer and Loewenthal with cultures from the omentum, and experiments with antibody formation in lymph nodes, suggest that the tissue cells themselves are capable of producing antibodies in the fully formed state.

The Response of the Antibody-forming Apparatus to Specific Stimuli

The Primary Response to a Specific Stimulus.—It will simplify discussion to consider first the response to the injection of an antigenic material directly into the blood stream. It will also be convenient to select an example in which the

antibody concerned is not present *ab initio* in the blood of the experimental animal employed. The production of flagellar agglutinins by the rabbit in response to a single intravenous injection of a killed suspension of *Salm. paratyphi B* affords a convenient illustrative example. Fig. 249 shows a typical reaction.

There is an initial induction phase, not shown in this particular diagram because the first reading after the injection of the antigen was taken on the fifth day. This induction phase usually lasts for 24 to 48 hours, by the end of which time agglutinin is usually detectable to very low titre (1 : 5 to 1 : 20). The titre then rises rapidly as shown in the figure, until about the 16th day. The time limits between which the maximum titre is usually attained may be put at about the 10th to 22nd day. It then falls, at first rapidly, then more slowly, agglutinins still being present in the blood 100 days after the inoculation. It will be noted that, in this chart, the titres are plotted as ordinates on a logarithmic scale, time as abscissæ on an arithmetical scale. Equal rises and falls in the curve represent not equal increments or losses of agglutinins but equal proportionate increases or decreases—for example, doublings or halvings of the amount present.

The Effect of Dosage.—As regards the relation between the dose of antigen administered and the amount of antibody produced, Table 81 sets out some figures for the particular case we are considering.

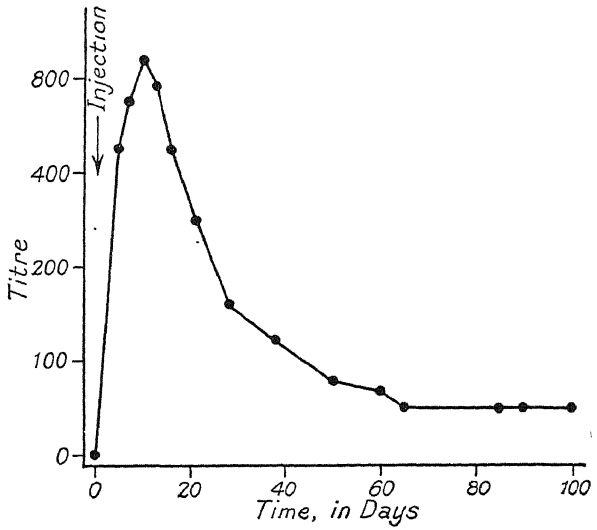


FIG. 249.

Production of agglutinins in rabbit after single injection of *Salm. paratyphi B.*

The minute dose of 10,000 bacilli per kilo. body weight, administered to rabbits varying from 1,380 to 3,750 gm., results in no detectable formation of agglutinins. When we increase the dose ten times we pass the threshold and obtain an average titre of 1 : 330. Over the range 10^5 to 10^8 bacilli a thousandfold increase in dose results in about a tenfold increase in titre—the critical student glancing at the numbers in the second column will not place too much reliance on the mean values recorded in the third. If we increase our dose still further we shall get still higher titres; but we shall be reaching the point where our inoculum attains an appreciable toxicity, and we shall, apart from this, be subject to the law of diminishing returns. However high we push our dose, up to the limit that will kill our rabbit, we are unlikely, after any single inoculation, to reach a titre of more than 1 : 10,000–1 : 20,000.

We may sum up in general terms by stating that there is a minimal threshold value, below which no response is obtained. Above this value the titre attained varies with the dose administered but in such a way that the increase in titre is relatively much smaller than the increase in dose required to produce it. Even-

TABLE 81

SHOWING THE MEAN VALUES OF THE HIGHEST TITRE OF FLAGELLAR AGGLUTININS IN SMALL GROUPS OF RABBITS INJECTED INTRAVENOUSLY WITH DIFFERENT DOSES (PER k.b.w.) OF *Salm. paratyphi B.*

No. of Bacilli injected per k.b.w.	No. of Rabbits tested.	Highest Titre. (Mean Value).
10^8	3	3,540
10^7	3	1,860
10^5	6	330
10^4	4	0 [1]

[1] = no agglutination at a dilution of 1 : 4.

tually a point is reached at which further increase in dose results in little or no increase in antibody production.

In connection with the problem of dosage, we may note one antigenic substance, the acetyl polysaccharide of the Type I pneumococcus, of which only very small doses induce an antibody response (Avery and Goebel 1933, Enders and Wu 1934). Thus Downie (1937) found an effective immunizing dose in mice lay between 1·0 and 0·01 μg., while doses of 100 or 1,000 μg. were ineffective.

Variations in Response to Different Antigens.—Fig. 250 shows the effect of injecting into an animal (a dog) a particular antigen (rat corpuscles) against which it possesses a normal antibody (hæmagglutinin).

It will be noted that the immediate effect of the injection is to cause a fall in the normal antibody titre, followed by a rapid rise of the type depicted in Fig. 249. This *negative phase* is a characteristic result of injecting any large amount of antigen into an animal that possesses the corresponding antibody in its circulating blood.

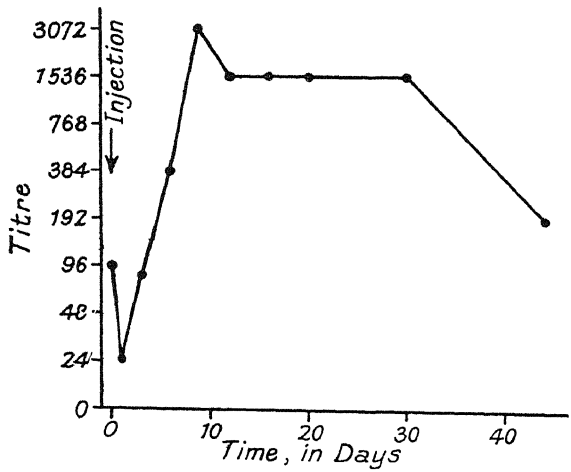


FIG. 250.

Production of hæmagglutinin in a dog after a single injection of rat corpuscles.
(After Hektoen and Carlson.)

The response to a single dose of an antigen in colloidal solution is illustrated in Fig. 251, which shows the course of precipitin formation in the rabbit following a single injection of horse serum.

There is a rather protracted induction period lasting for some 8 days, followed by a rise in antibody titre to a maximum about the 16th day and then a slower fall, till precipitin ceases to be detectable about the 37th day. It should be noted that this curve is not comparable, at a mere glance, with those given earlier in the chapter since the titres are plotted as an arithmetical not as a geometrical or logarithmic series. To make the

curve comparable with those describing agglutinin or hæmolysin production the logarithms of the number of precipitin units would have to be plotted as ordinates.

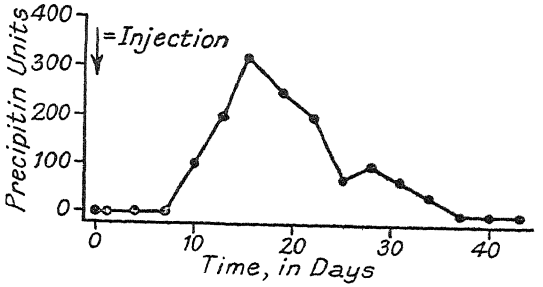


FIG. 251.

Precipitin production: response of a rabbit to single injection of horse serum.
(After Dean and Webb.)

The Response of the Antibody-forming Apparatus to the Simultaneous Injection of Several Antigens.

It is clear that the capacity of the antibody-forming apparatus must have some limit. The question at issue is at what stage that limit is reached. Will antibodies be formed as readily against two or three or n antigens, all injected at the same time, as against a single antigen? The answer is of considerable practical interest, since mass immunization against a multiplicity of diseases could be envisaged more cheerfully if syringes could be loaded on the blunderbuss principle. The evidence is conflicting.

There are records (Benjamin and Witzinger 1912, Huntoon and Craig 1921) which suggest that the injection of more than one antigen, simultaneously or in rapid succession, lessens the production of one or other of the corresponding antibodies. Corrigan (1925), and Hektoen and Boor (1931), on the other hand, have recorded results which indicate a far less restricted range of activity. The latter observers immunized rabbits by repeated intravenous injections of mixtures containing large numbers of different antigens, each in a relatively pure state. These included hæmoglobin from the ox, cat, dog, hog, horse, man, sheep and turkey, horse pseudoglobulin, serum albumin from chicken and man, egg albumin and other constituents. One of the mixtures contained 14 different antigenic constituents, the other 35. Precipitins were produced against almost all the antigens injected, and in most cases to high titre. In the particular case of antitoxins the careful work of Glenny and his colleagues (Glenny 1925*a*, Glenny, Hopkins and Waddington 1925, Glenny and Waddington 1926, 1928) makes it clear that the simultaneous injection of several antigens may considerably reduce the titre of anti-

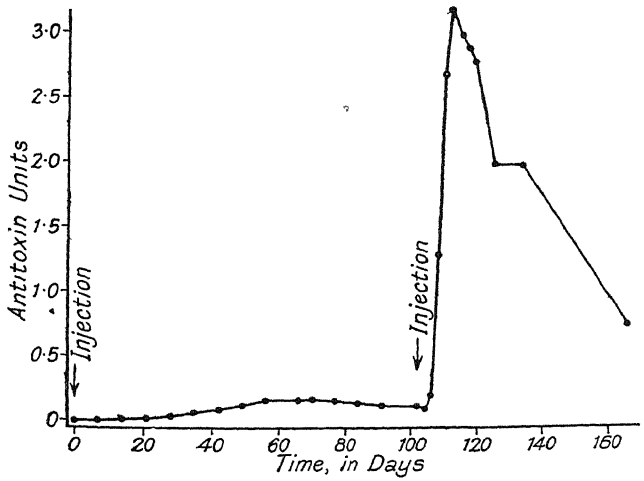


FIG. 252.

Antitoxin production in the horse: showing difference between primary and secondary response.
(After Glenny and Südmersen.)

toxin obtained. It seems likely, as Glenny suggests, that this " crowding out " effect is particularly prone to occur when any one antigen is present in great excess.

Bjorneboe (1941) immunized rabbits with vaccines containing from one to eight types of pneumococcus, which were given every second day for 8 months; the antibody production per type was the same for mixtures up to three types, and became progressively weaker as the number of antigens increased. It is noteworthy that when the " ceiling " values of serum antibody were attained, all the serum globulin appeared to consist of antibody. The separate antitoxic response of rabbits to mixtures of diphtheria, tetanus and staphylococcal toxoids (Ramon and Richou 1940) and of diphtheria and staphylococcal toxoids given intranasally (Sato and Kodama 1940) has been found to be similar to that following injections of the single antigens.

In man, a combination of two or more antigens is reported by some to have a synergic effect, each antigen being more effective in combination than alone. For example, synergic effects are recorded for mixtures of diphtheria and tetanus toxoids, with and without typhoid vaccines (Sacquepée, Pilod and Jude 1936*a*, *b*); for staphylococcal and diphtheria toxoids (Kodama, Sato and Hata 1940); for tetanus toxoid and typhoid vaccines (Ramon 1936, Maclean and Holt 1940; see also Grasset and Girdwood 1940, Fraser *et al.* 1943); for diphtheria and tetanus toxoids (Bigler and Werner 1941), and for these two toxoids and *H. pertussis* vaccine (Lapin, 1942). Neither synergic effect nor depression of antigenic stimulus was found in such mixtures as vaccinia and diphtheria toxoid (Stern 1935), diphtheria and tetanus toxoids (Jones and Moss 1937, Peshkin 1941), diphtheria toxoid and *H. pertussis* vaccine (Kendrick 1942, Sauer and Tucker 1942). With a mixture of *H. pertussis* vaccine, tetanus and diphtheria toxoids, Miller and Saito (1942) record a depression of the tetanus antitoxin response.

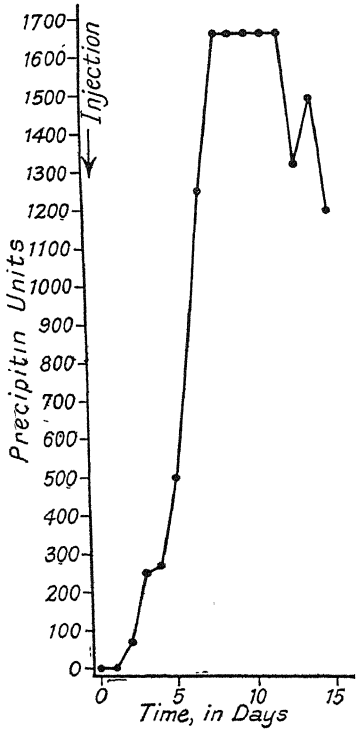


FIG. 253.

Precipitin production: response of a previously sensitized rabbit to a single injection of horse serum—secondary response.

(After Dean and Webb.)

With optimal doses of each constituent, and a total inoculum which is not unduly large, it seems probable that an effective response can be obtained against a reasonable number of different antigens, even if the titre of some or all of the corresponding antibodies is relatively reduced, and that in some cases there is a synergic effect in antigenic mixtures. From the practical point of view the problem will clearly differ according as we are attempting to induce an active immunity in man or animal, or to produce a high titre serum for passive immunization. In the former case a reduction in the antibody titre attained might be of minor importance, in the latter it would be a grave disadvantage. Each case, as it arises, should be subjected to experimental trial.

The Difference in Response to Primary and Secondary Stimuli.—Having considered the response of the antibody-forming apparatus to an initial injection of antigen we may inquire how it responds to repeated stimuli, and it will be convenient to take as our first instance the response of a horse to the injection of diph-

theria toxin, selecting an animal whose blood, at the time of the initial injection, is devoid of antitoxin. This particular reaction has been studied in great detail by Glenny and his colleagues (Glenny 1925*b*, 1931, Glenny and Südmersen 1921) and forms the basis of his insistence on the fundamental difference between a primary and a secondary response. Fig. 252 shows a typical example.

The primary injection is followed by an induction period of some 14 days, after which there is a very slow rise in the antitoxin content of the blood, reaching a low maximum of 0·16 units per ml. between the 9th and 10th weeks, and sinking slowly to 0·11 units by the middle of the 15th week, at the time of the second injection. The response to this second injection is of an altogether different character. Within 4 days a distinct rise in the titre of antitoxin is observable, and the curve then rises steeply to a maximum of 3·2 units per ml. on the 10th day, followed by a fairly rapid decline. It may be added that with repeated and increasing doses the antitoxin content of a horse's serum may be raised to well over 1,000 units per ml.

This difference in response to primary and subsequent injections of the same antigenic material is not limited to the production of diphtheria antitoxin, though it is particularly well marked in this case. Fig. 253, showing the response to an injection of horse serum in a rabbit which had previously been injected with that antigen, but whose precipitin titre had since sunk to zero, may be compared with Fig. 251 above.

The same holds true, in regard to the comparative titre attained, though not in regard to the prolonged induction period after the primary stimulus, when the antigenic material is particulate, as in the case of a bacterial suspension. Table 82 sets out the primary and secondary response of rabbits to the intravenous injection of 10^5 *Salm. paratyphi B* per kilo. body weight. It will be noted that the titres given are not the maximum attained but the average titre during the 50 days after inoculation. This value was selected because the curve of agglutinin titre after a secondary inoculation tends not only to rise higher than after a primary injection, but to remain high over a longer period.

TABLE 82

SHOWING THE AVERAGE TITRE OVER A PERIOD OF 50 DAYS AFTER PRIMARY AND SECONDARY INJECTIONS INTO RABBITS OF 10^5 *Salm. paratyphi B* PER K.B.W.

Rabbit.	Primary Average Titre.	Interval between 1st and 2nd Injections.	Secondary Average Titre.
R11	280	147 days	2,720
R19	37	147 ,,	1,720
R25	130	147 ,,	430
R30	130	133 ,,	1,230
R31	91	133 ,,	1,230
R33	110	133 ,,	2,010

The difference between the primary and secondary response is sufficiently obvious and needs no comment. With very small doses there may be no detectable primary response, antibodies appearing first after the second or later injection of a bacillary suspension. An illustrative example is given in Table 83.

Burnet (1941) has emphasized a distinguishing feature of the secondary response

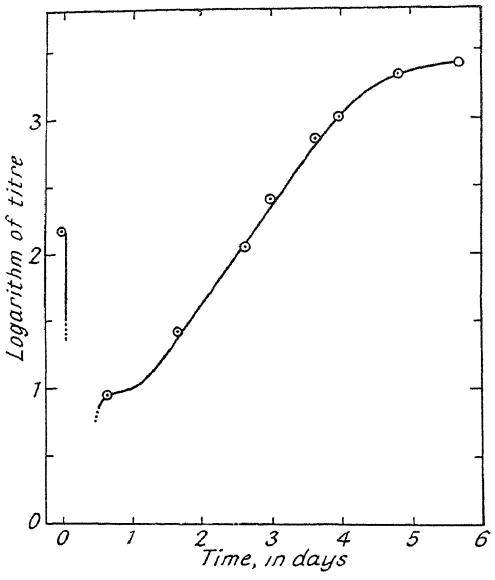


FIG. 254.

The secondary response of an immune rabbit to a single large intravenous dose of staphylococcal toxoid.

(After Burnet 1941.)

that is especially obvious when the stimulating dose of antigen is given intravenously. He studied the secondary response to staphylococcal toxoids, herpes virus and a bacteriophage, and in each case found that after a delay of 1 to 2 days, the antibody content of the serum increased exponentially with regard to time, until the maximum level was approached, when the rate of increase in antibody-content gradually diminished to a low value. In Fig. 254, for example, where the staphylococcal antitoxin content of the serum is plotted logarithmically, the curve is practically linear from the 2nd to the 4th day, after which it tends to the horizontal.

The tendency for the antibody titre to be maintained at a high level for a longer time after secondary and subsequent responses than after a primary response is illustrated in Fig. 255, showing the response of a rabbit to three widely spaced injections of *Salm. typhi*, Fig. 256, showing the response of an uninoculated person to an injection of typhoid vaccine, and Fig. 257, showing the response of a previously inoculated person to the same antigenic material.

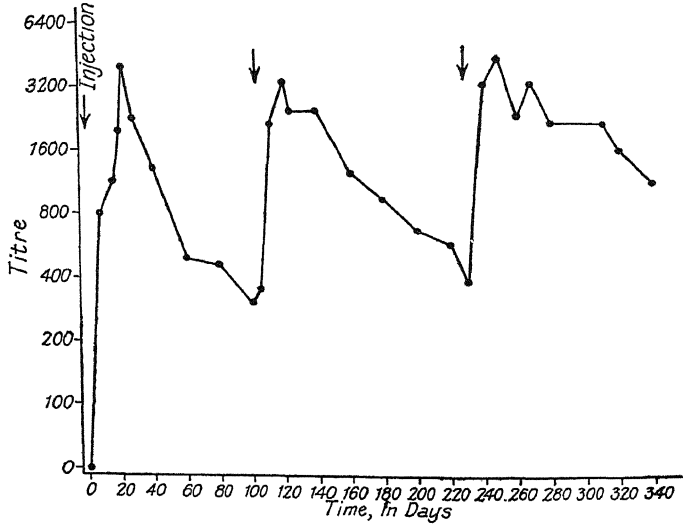


FIG. 255.

Agglutinin production : response of a rabbit to widely spaced injections of *Salm. typhi*.

TABLE 83

SHOWING THE HIGHEST TITRE ATTAINED AFTER 1ST, 2ND AND 3RD INJECTIONS OF 10^1 *Salm. paratyphi B* PER K.B.W.

Rabbit.	Highest Titre after 1st Injection.	Interval between 1st and 2nd Injections.	Highest Titre after 2nd Injection.	Interval between 2nd and 3rd Injections.	Highest Titre after 3rd Injection.
R16 . . .	0	28 days	8	92 days	40
R32 . . .	0	28 „	32	92 „	319
R46 . . .	0	28 „	7	92 „	20
R47 . . .	0	28 „	0	92 „	0

It must not be supposed that the differences between a primary and secondary response are always so clear-cut as those depicted here. The curves may, however, be regarded as giving a true picture of the kind of differences observed. It will be noted in Figs. 255 and 256 that the primary response may be quite brisk and result in a high titre of agglutinins. This, for instance, is the usual experience as regards the production of H agglutinins in response to the injection of an optimal dose of flagellated bacilli.

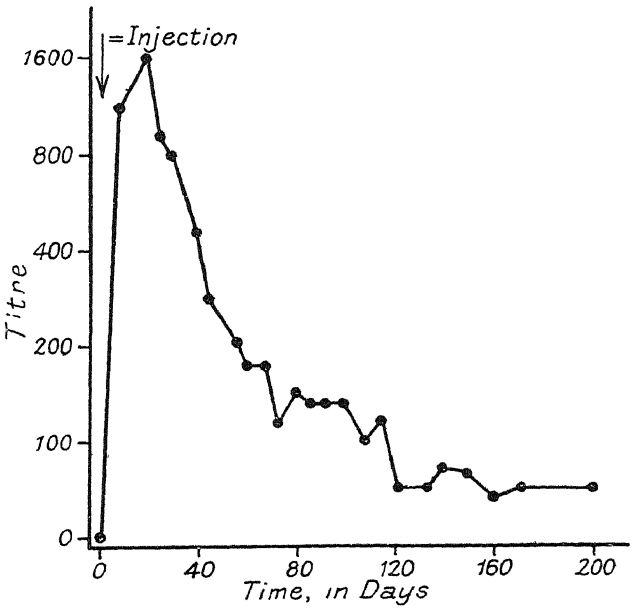


FIG. 256.

Agglutinin production: response of an uninoculated person to a single injection of typhoid vaccine.

We have noted that increases in the dose of antigen administered at a single injection are subject to the law of diminishing returns: in just the same way the repetition of injections, beyond a certain number, is usually found to produce less and less effect with each successive dose. When the titre of a particular antibody has been forced up to a certain level, which varies widely with the nature of the antigen employed and the responsiveness of the animal injected, it becomes impossible to induce any further rise in the concentration of antibody in the circulating blood.

Fig. 258 shows the effect of repeated subcutaneous injections of typhoid bacilli in the rabbit (Goldberg 1901). It will be noted that each successive injection raises the agglutinin titre, but that the increments tend to become proportionately smaller and smaller, the last injection on the 35th day producing a relatively trivial effect.

The importance of the secondary response (the " injection de rappel " of the French workers, the " boosting dose " of the American), in practical immunology must be emphasized. The primary stimulus apparently " conditions " the antibody-forming apparatus in such a way that a later stimulus from the same antigen induces a rapid and copious outflow of antibodies into the blood.

The study of antitoxin response to the immunization of man by diphtheria toxoid (see, for example, Jones 1938, Chesney 1939, and Chapter 61) and tetanus toxoids has thrown some light on the time relations of the primary and secondary responses. We may summarize the findings as follows: Both the serum antibody response and the " conditioning " produced by a single dose of toxoid may be low; large single doses are better than small. Two primary doses are better than one and the response is more effective as the interval between increases from 2 to 6 weeks. The response to a second dose at 6 weeks often has the marked character of a secondary response. Nevertheless, the " conditioning " process has not reached its maximum, for if a third dose is given 2 to 12 months later, the antibody level rapidly rises to a new and higher peak, and declines proportionately slowly. The same applies to immunization against tetanus (see Bergey 1934, Sacquepée and Jude 1937, Boyd 1938, Sacquepée, Pilod and Jude 1938, Marvell and Parish 1940, Peshkin 1941, Evans 1943). Schutze (1941) records an experiment in which mice were immunized with two doses of typhoid vaccine, one group at an interval of one week, a second at an interval of four weeks. The titres in the second group were 50 per cent. higher than in the first.

It is a common observation that the response to an antigen of an animal whose serum already contains a low level of specific antibody is greater than that of animals without serum antibody. Jensen (1937), for example, employs the relation between the two to group children in an ascending scale of immunizability to diphtheria according to the antitoxin levels in their blood (see also Flood 1930). However, absence of circulating antibody does not necessarily indicate absence of a " conditioned " antibody-forming apparatus. Thus, marked secondary responses to diphtheria toxoid can be evoked in children with little circulating antitoxin, 5 years after immunization (see, for example, Volk, Bunney and Tripp 1943).

The Enhancement of Response to an Antigenic Stimulus.

The possibility of enhancing the antibody response to a given dose of antigen has been explored by immunologists for many years. Among the numerous procedures devised to this end we may note two kinds—enhancement of response by variation in the mode or route of inoculation, and enhancement by the addition of adjuvant substances to the antigen. With regard to the route of immunization, it is generally held that antigens in the form of large particles, like bacteria or animal erythrocytes, are most effective when given intravenously (see, for example, Marinelli 1937) while soluble antigens are best given into the tissues. If a soluble antigen is attached to coarsely particulate matter, it may be most effective by the intravenous route (Freund and Bonanto 1941). A probable cause of the relative inefficacy of intravenously injected soluble antigens is their rapid elimination from the body. The same factor probably diminishes the stimulus of antigens injected into the tissues, for most of the procedures that increase antigenicity appear to act by hindering the release of antigen from the injection site.

Variation in the volume of the inoculum.—The experiments of Hartley (1935) indicate that the total volume of an immunizing injection, as well as its content in antigenic material has a significant influence on the antibody response. A series of guinea-pigs were injected with a constant dose of diphtheria toxoid made up in a total volume of 0·5 ml. or 5·0 ml of saline. In repeated tests it was found that the animals receiving the larger volum

gave a far better immunizing response. Timmerman and Brandwijk (1936*a*, *b*) confirmed Hartley's results. Schmitz (1938), however, repeated the test in medical students, and found no difference either in antitoxin response or in the rate at which antitoxin disappeared from the blood.

Dispersal of the inoculum through the tissues.—A stimulation of response resembling that of Hartley (1935) may be achieved by sub-dividing a dose of antigen into several smaller doses and injecting each into different sites in the animal. The response is greater than that produced by the whole dose administered singly (Ramon, Lemetayer *et al.* 1937, Miles and Pirie 1939).

The addition of adjuvants.—Adjuvants are usually intended for injection into the tissues, and are for the most part mildly irritant substances. Alum is one of the most widely used adjuvants. It was introduced by Glenny and his colleagues (Glenny *et al.* 1926) for improving the antigenicity of diphtheria toxoid, but has since been applied to other toxoids and to bacterial vaccines like those of *H. pertussis* (Chapter 74). Aluminium hydroxide is also effective (Hektoen and Welker 1933). Other successful adjuvants are lanoline in oil, cholesterol, salts of calcium and magnesium (Ramon *et al.* 1935); tapioca (Ramon 1937, 1939, Schmidt and Steenberg 1936); bacterial products like typhoid vaccine (Ramon 1936), staphylococcal toxin (Burky 1934, Swift and Schultz 1936); tubercle bacilli (Freund and McDermott 1942); serum containing heterophile antibodies (Kalinin *et al.* 1935). Both Glenny and Ramon attribute the action of alum and tapioca respectively to the stimulation of tissue lesions, from which the antigen is slowly liberated, thus providing a prolonged and continuous antigenic stimulus.

The lesion produced by a single injection of alum-precipitated diphtheria toxoid is apparently the chief source of antigen for some time after its formation. Thus Blagowechensky (1938) excised the lesion made by alum-toxoid at varying intervals after its formation in the skin of guinea-pigs and found that, within a period of 10–20 days, the degree of immunity reached depended almost entirely on the length of time the lesion had remained in the body.

The adjuvant effect has also been attributed to the high absorptive capacity of the substances employed. Aluminium hydroxide, for example, will absorb proteins *in vitro* (Hektoen and Welker 1933, Cohen and Mosko 1943, Holford, Ludden and Stevens 1943). Faragó and Ujhelyi (1942) injected groups ofguinea-pigs with diphtheria toxoid alone, with toxoid and $Al(OH)_3$ in the same injection sites, and with toxoid and $Al(OH)_3$ in different sites. The average antitoxin responses in units per ml. after four weeks were 0·005, 0·4 and 0·02 respectively. In the last group the toxoid was apparently absorbed by the separately formed $Al(OH)_3$ lesions, since lesions were antigenic when transplanted into normal guinea-pigs (see also Westwater 1940). It is in this case impossible to decide whether the *in vivo* absorption of toxoid was due to the $Al(OH)_3$ or to the cells of the lesion it induced. Ramon (1936, 1938) holds that the inflammatory response to the adjuvant is paramount in the adjuvant effect (see also Schmid 1938*a*) and on this basis devised a method of " concentrated immunization," in which small amounts of antigen are injected 4 or 5 times daily in the same site for periods of up to three weeks. By this means an inflammatory lesion is rapidly produced, into which toxic antigens may be injected without inducing a general toxæmia. In all cases the final antigenic response to this procedure was greater than that to the same total dose given in one injection. The inflammation was non-specific in its action, the response to tetanus toxin, for example, being increased by injection into a site inflamed by another antigen (Ramon, Lemetayer *et al.* 1937).

Fats are employed as adjuvants, and also for the purpose of modifying the toxicity of certain antigens (see Chapter 70). Ramon, Richou and Staub (1937) emulsified living *C. diphtheriæ*, *Staph. aureus* and *B. anthracis* in lanoline, and on subcutaneous injection obtained in the first two cases good antitoxic immunity, and with *B. anthracis* a high antibacterial immunity. Since the bacilli alone had no such effect they concluded that the lanoline allowed a local multiplication of the bacilli, production of toxins, a detoxication of toxins by the animal tissues without destruction of antigenicity, and a gradual release

of antigen from the injection site. Freund and his colleagues used lanoline to improve the antigenicity of heat-killed tubercle bacilli, and found that the size of the immune response depended on the size of the local lesion, which consisted of a non-necrotic monocytic reaction with fibrous encapsulation. The oil-tubercle bacillus mixture itself proved to be an active adjuvant for other antigens—horse serum, avirulent pneumococci, diphtheria toxoid and *Salm. typhi* (Freund *et al.* 1940, Freund and McDermott 1942, Freund and Bonanto 1944; see also Landsteiner and Chase 1942, Friedewald 1944).

We may say then that the antibody response to certain antigens injected into the tissues may be increased by methods which appear to diminish the rate of antigen release from the injection site. In the light of our discussion on the formation of antibody in the skin and lymph glands we cannot exclude the possibility that the local tissue reaction to some adjuvants includes cells capable of antibody formation, or that adjuvants act by increasing the number of antibody-forming centres in other parts of the body. In Hartley's (1940) experiments, for example, antibody was apparently formed in a predominantly macrophage nodule induced by $Al(OH)_3$ (p. 1102). Moreover, bacterial substances of the kind that have an adjuvant action on other antigens have also been shown to stimulate the formation of the germinal centres in lymphoid tissue (see p. 1105).

The Negative Phase.—Just as the injection of a considerable dose of a particular antigen into an animal that possesses a corresponding normal antibody may result in a temporary decrease in the concentration of that antibody in the circulating blood, the injection of a large dose of antigen into an animal that has already developed a certain concentration of antibody in response to earlier injections may be followed by a well-marked negative phase, which is usually followed in its turn by a rise in titre above the previous level. An illustrative example is afforded by the response of an immunized rabbit to two injections of horse serum, given intravenously at an interval of 5 days (Fig. 259). The importance of avoiding this negative phase in therapeutic immunization has been emphasized particularly by Wright and his co-workers (Wright 1909), though others maintain that in these circumstances there may be little diminution in the total immunity.

Schütze (1939), for example, infected mice with a dose of *Salm. typhi-murium* sufficient to kill 50 per cent. of the animals in about 14 days. The injection of four substantial daily doses of a specific vaccine of proved efficacy, beginning on the second day of the disease, did not increase the average mortality or the average time to death of the mice that died; in some experiments the vaccine appeared to save some of the mice.

Here the immunization of animals with an already established infection increased neither the severity nor the fatality of the disease. It must be remembered, however, that even though similar results were to follow tests of this kind with other infections, there may be conditions in which the negative phase is deleterious. In practice we are often concerned with the dangers of immunization of a herd that has been exposed to an infection, and that contains individual members who are invaded, but not yet infected, by the causal organism. In this case, a disturbance of the immune response may affect another perhaps more delicately balanced issue, namely the destruction or establishment of the invading organisms in the tissues of the host; and immunization might be followed by an increase of morbidity within the herd.

The diminution of circulating antibody in the negative phase, however, has implications other than a temporary depression of specific immunity in the animal. The newly injected antigen presumably combines with the antibody, and it is pertinent to inquire whether the antigen is modified by its union with antibody

Olitzki (1935) succeeded in suppressing the antigenic stimulus of a bacterial vaccine

in rabbits, either by preliminary lysis of the bacteria and saturation of the contained antigens with homologous antibody, or by a preliminary saturation of the surface antigens of the whole bacteria with antibody and a simultaneous injection of an excess of antibody into the test animal. This " blanketing " effect of antibody on antigenicity is of considerable importance in such procedures as immunization against diphtheria by the " combined method," in which antitoxin and toxoid are given at the same time, the one to provide an immediate passive immunity, the other as the primary stimulus for a more lasting active immunity. It is clearly desirable that the primary stimulus should be effective as early as possible in order that active immunity is established before the passive immunity has disappeared. It is, of course, impossible to measure the active antibody response to the primary dose of toxoid in the presence of the high concentration of passively administered antibody, but its effect on the antibody-forming apparatus can be estimated by the magnitude of the response to a second injection given some time later. If it is of the magnitude of a " secondary response," we may conclude that the primary dose has been effective. The results of a number of tests in man and animals are to some extent conflicting, but there is little doubt that if large doses of antitoxin are given, the toxoid may be partly blanketed and the development of active immunity delayed and inhibited to a variable degree (see Downie *et al.* 1941). The problem is fully discussed in Chapter 61. but we may note here that the blanketing effect is less apparent when relatively insoluble antigens are used, alum precipitated toxoid being more effective than fluid toxoid (Gundel and König 1938, Phair and Root 1942), and that by careful spacing of the subsequent doses of toxoid, the interval between declining passive and increasing active immunity may be made relatively small. The same considerations hold in combined active and passive immunization with tetanus toxoid and antitoxin (Ramon 1938, Cooke and Jones 1943, Gold and Backers 1943).

The Response of the Antibody-forming Apparatus to the Administration of Antigens otherwise than by Injection into the Tissues.

In defining the properties of an antigen on p. 194, it was stated that it should stimulate the formation of an antibody when introduced *parenterally* into the body. This caveat was included in the definition because administration *via* the intestinal tract does not ensure the passage of the antigen to the tissues in an unaltered and effective form. It may be broken down by the digestive enzymes. If it is not acted on by them it may never pass through the intestinal mucosa. Whether, in fact, any particular antigenic material administered by this route reaches the tissues in an unaltered form can only be determined by direct experiment. The available evidence suggests that some bacterial antigens, but not all, induce the formation of specific antibodies when administered by the mouth.

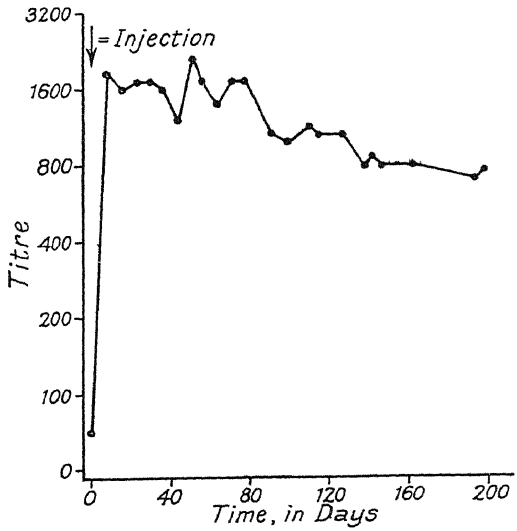


FIG. 257.

Agglutinin production: response of a previously inoculated person to a single injection of typhoid vaccine.

Thus, the administration *per os* of killed suspensions of flagellated bacilli of the typhoid-paratyphoid group results in the production of somatic (O) agglutinins, but flagellar (H) agglutinins are either not produced at all, or only to low titre (Pijper and Dau 1930, Greenwood, Topley and Wilson 1931). The same difference between the formation of H and O agglutinins appears to hold in the case of the cholera vibrio (Pfeiffer and Lubinski 1930). Dysentery bacilli, which are non-flagellated, induce the formation of somatic agglutinins when administered by the mouth (Kanai 1921, Otten and Kirschner 1927); and it is probable that analogous effects are produced by many other bacterial cells (see for instance Ross 1926, 1930, 1931, 1932, 1934, Downs and Bond 1937, Moor and Brown 1937, Torikata and Imaizumi 1938). It appears that the Vi antigen of the typhoid bacillus is ineffective by the oral route. Guarnacci (1939), for example, obtained low H and O titres in 68 per cent. of 227 persons given an oral vaccine of a (Vi + O) strain, but no Vi agglutinins.

In all cases it would seem that administration *per os* is far less effective than inoculation into the tissues, in the sense that much larger doses have to be given and the antibody titre attained is usually lower.

As regards other routes of entry, Stillman (1927, 1930) has shown that protective antibodies are produced in rabbits after the administration of living pneumococci by inhalation, but here we may be dealing with an active infection and consequent tissue invasion by living bacterial cells.

The intranasal application of diphtheria toxoid, by instillation, insertion on pledgets

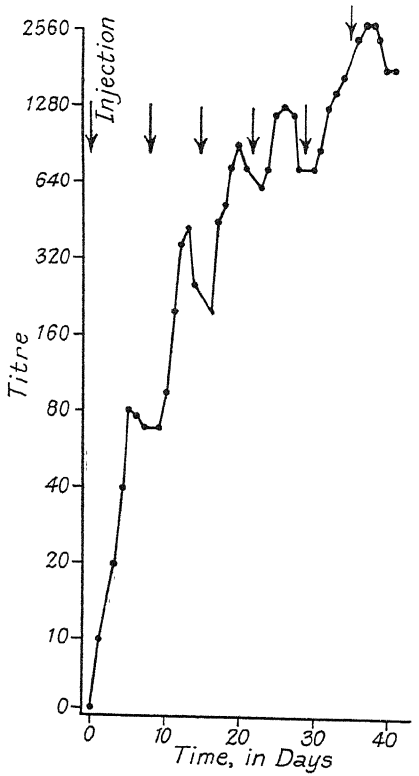


FIG. 258.

Effect of repeated injections of *Salm. typhi* on agglutinin production in a rabbit. (After Goldberg.)

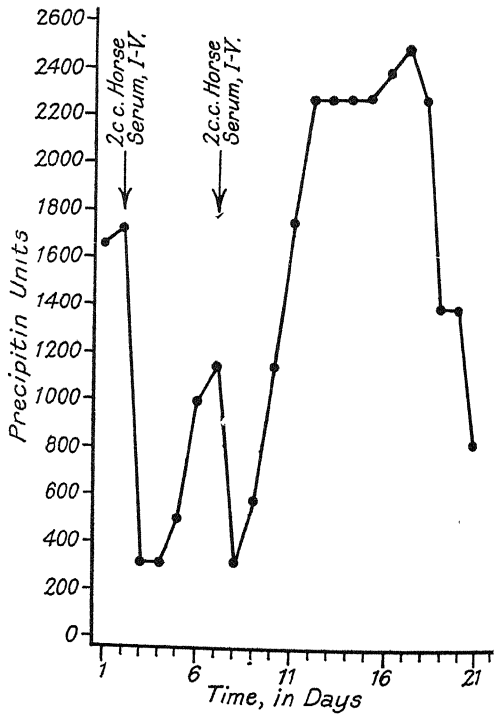


FIG. 259.

Precipitin production: effect of intravenous injection of horse serum into an immunized rabbit. (Dean and Webb, 1928.)

of cotton wool, or as an atomized mist (Jensen 1937, Bousfield and King-Brown 1938, Fraser *et al.* 1940, Kodama, Sato and Hata 1940, Sato and Kodama 1940, Sordelli *et al.* 1942) acts as an effective stimulus to antitoxin formation, though less effective than injection into the tissues. Sordelli and his colleagues (1943) tested a number of antigens by the nasal and subcutaneous routes, and noted that *Salm. typhi* was ineffective by the nose; and that compared with subcutaneous injection a *Br. suis* endoantigen was less effective, staphylococcal toxoid equally effective, and tetanus toxoid more effective by the nasal route. Antibody response to intranasal vaccines of the influenza virus has also been demonstrated (see Chapter 88).

It seems justifiable to conclude that the administration of non-living antigenic material by various routes other than directly into the blood stream or tissue spaces will often result in antibody production, but that none of these alternative routes is usually either so certain or so effective.

The Nature of the Substances that stimulate Antibody Production.

In the preceding discussion we have been concerned with antigens of the chemically complex type, ranging from purified proteins to crude suspensions of bacteria or other cells. We have discussed in Chapter 7 the validity of the hypothesis that only proteins or protein-containing substances can induce an antibody response when injected into the tissues. It is clear that protein-free derivatives of fully antigenic substances not only act as haptens, but in some cases are themselves fully antigenic. There can be no doubt of the validity of Landsteiner's broad distinction between complete antigens and haptens, or of the association between decreasing chemical complexity, or molecular size, and a progressive loss of antigenic function; but it is not possible to predict at what stage in the degradation of a given antigen the complex will lose its full antigenic powers. In this connection it is instructive to consider the main type-specific antigen of the pneumococcus.

All the early studies on the purified polysaccharide components isolated from various bacteria, and in particular from pneumococci, suggested that these substances had the properties of haptens, and induced no immunizing or antibody-forming response when injected into the tissues. But observations have since accumulated which are difficult to reconcile with such a view.

Schiemann and Casper (1927) reported that the purified specific polysaccharide of the Type I pneumococcus, when injected into mice, produced an active immunity against living Type I pneumococci, but not against other types. They noted that this immunity was induced by the injection of small, but not of larger, doses. Schiemann (1929) recorded the immunization of mice by the injection of Type II pneumococcal polysaccharide, and noted that the blood of these immunized mice contained protective antibodies; though he was unable to demonstrate antibody production in rabbits. Francis and Tillett (1930) recorded observations suggesting that persons who were given repeated intradermal injections of the purified polysaccharide of Type I, II or III pneumococcus, developed a specific local response which took the form of an immediate wheal and erythema; and that this response was associated with the appearance of specific antibodies in the blood. Similar observations have been reported by Finland and Sutliff (1932) and by Zozaya and Clark (1932). Zozaya and Clark (1933) recorded the immunization of mice with polysaccharide fractions from Types I, II and III pneumococci, and noted that small doses were more effective than larger ones; but their results were irregular except when the polysaccharides were adsorbed on collodion particles or on carbon.

Later studies by Avery and Goebel (1933) have thrown further light on this question. They were able to show that the Type I pneumococcal polysaccharide, as it occurs in the bacterial capsule, possesses acetyl groups. These groups are lost during the separation

and purification of the polysaccharide by the methods which have been commonly employed. The acetylated form was found to induce active immunity in mice, when it was administered in small, but not in large doses. It did not induce antibody production in rabbits. Analogous results have been recorded in relation to other bacteria. For example, in *Salm. typhi* and *Br. melitensis* the lipopolysaccharide O-substance is fully antigenic; whereas in *Sh. shigæ* the antigenicity of the O-substance is lost when the association between the polypeptide and lipopolysaccharide moieties of the molecule is destroyed (Chapter 8).

The Response of the Antibody-forming Apparatus to Active Infection

The examples given above all refer to the response of the antibody-forming apparatus to the experimental injection of non-living antigenic substances. We may close this section with a few examples of its response to natural infections of the clinically obvious type. For simplicity we may confine our attention to acute infections, noting, however, that in the case of subacute and relapsing diseases, such for instance as tuberculosis, there is evidence that the antibody titre fluctuates as the infection varies from quiescence to activity.

As a typical example of an antibody curve during the course of an acute febrile infection we may take some of the numerous observations that have been made on patients suffering from typhoid fever. Figs. 260 and 261, constructed from data recorded by Jörgensen (1905), show the fluctuations in typhoid agglutinins during a typical attack of the disease, and during the terminal stage of a primary attack and the two relapses which in this case immediately followed it. The clinical course of the disease is, in each case, indicated by the fluctuations in temperature. It will be noted that, in the second of these cases, the agglutinin response during the primary attack was unusually feeble.

The Response of the Antibody-forming Apparatus to Non-Specific Stimuli.

Since the serum antibodies react specifically with their corresponding antigens it is natural to suppose that they will be formed *de novo* only in response to the specific stimulus provided by the introduction into the tissues of the antigen concerned. In making this supposition we must, however, be very careful to limit its implications. We must recollect, as has been emphasized in Chapter 8, thatwe are concerned with chemical substances, and not with particular species of bacteria or particular types of cell. We know that certain antigens are shared by several bacterial species; and the production, in response to the introduction into the tissues of one bacterium containing antigens a and x, of antibodies acting on another bacterium containing the antigens b and x is, in so far as it depends on the production of antibody X, a strictly specific response in the only sense with which we are here concerned. With these limitations, our supposition is in accord with all the recorded facts. The available literature contains no instance in which the introduction into the tissues of a substance of known chemical constitution has led to the production *de novo* of an antibody reacting specifically with a second substance, the chemical constitution of which bears no relation to that of the first.

If we turn to the production of a normal antibody in increased amount, a rise in the titre of an antibody previously produced in response to a specific stimulus, or the reappearance of such an antibody after the titre has fallen to zero, it is clear that our supposition no longer holds on *a priori* grounds. There would be nothing remarkable in an antibody-forming apparatus that had once produced, and possibly stored, a particular antibody, turning that antibody into the circulation anew, or in greater amount, in response to some non-specific stimulus which increased the activity of the apparatus without directing it into any particular channel.

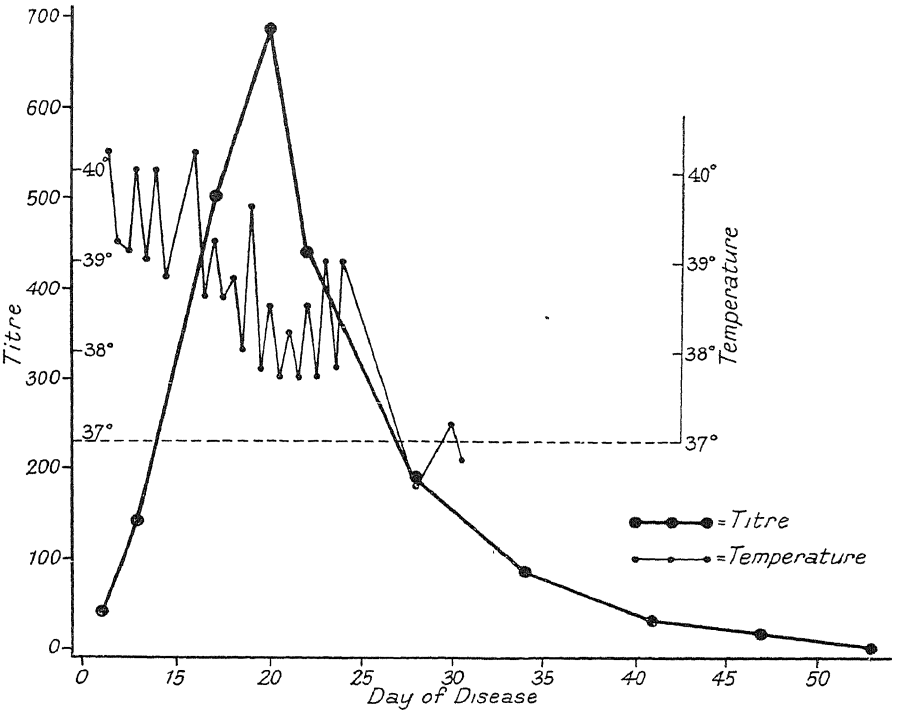


FIG. 260.

Rise and fall of agglutinins in blood of patient during an attack of typhoid fever.
(After Jörgensen.)

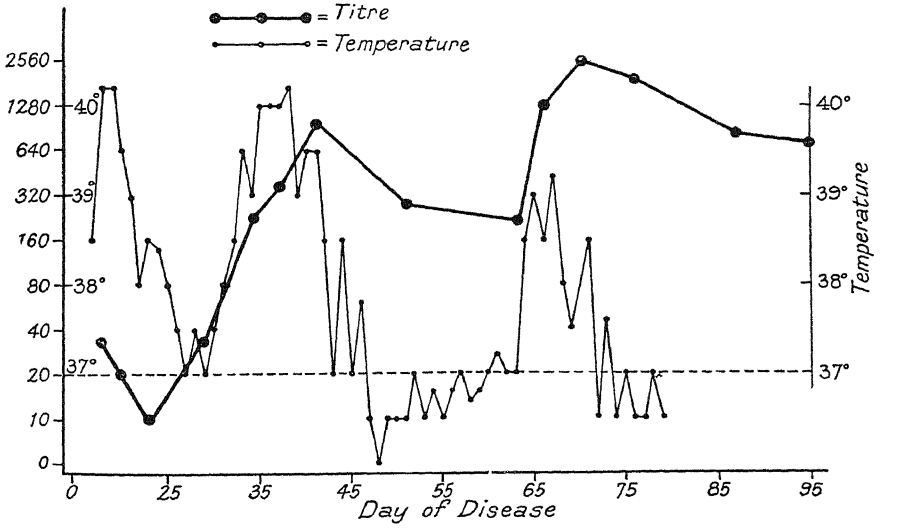


FIG. 261.

Fluctuations of agglutinins in blood of patient during attack of typhoid fever, followed by two relapses.
(After Jörgensen.)

Whether or not this type of non-specific stimulation occurs can be settled only by empirical trial.

In this connection we may note a terminological confusion which has sometimes arisen in the literature. The production, in response to an antigenic stimulus, of an antibody that has been produced in the tissues on some previous occasion, is often referred to as an *anamnestic reaction* (*anamnesis*—recollection). Sometimes this term is used in connection with the type of non-specific stimulus to which we have just referred—the tissues are stimulated to general activity, in which is included the renewed production of a particular antibody that had been formed at some time in the past. Sometimes, perhaps more accurately, it is employed in reference only to those instances in which the particular chemical stimulus concerned formed part of the earlier experience of the antibody-producing cells. In this limited sense it is clearly synonymous with the secondary response, in the sense defined above, and has no reference to the problem with which we are here concerned.

Several observers have recorded a stimulation of antibody production as the result of excessive or repeated bleeding.

Friedberger and Dorner (1905) stated that the withdrawal of considerable amounts of blood, either shortly before an immunizing injection, or within a few days thereafter, had a definitely stimulating effect on the subsequent antibody production. Hektoen and Carlson (1910), in the experiments already referred to, found that bleeding a dog dry during the initial latent stage of active immunization and immediately transfusing it with the blood of a normal dog appeared to increase the production of antibodies. Hahn and Langer (1917) have reported experiments in which they observed a marked increase in agglutinin titre in 4 rabbits as the result of repeated daily bleedings. Trommsdorf (1921) has recorded an increase in agglutinins for the diphtheria bacillus as the result of daily bleedings. Other workers (Klinger 1918, Landau 1918) have however failed to confirm these results. It may be noted that an opposing factor is brought into play when an immunized animal is subjected to massive repeated bleeding. The mere withdrawal of considerable quantities of blood would, of itself, tend to lower the titre of the circulating antibodies if the antibody-forming apparatus did not share in the subsequent activity of the hæmopoietic tissues. That immunized animals may suffer extensive bleedings without any detectable and lasting fall in antibody titre has long been known (Roux and Vaillard 1893).

Monaco (1939) immunized rabbits with a heat-killed typhoid vaccine, and, 4 months after the last injection, exposed them for 30 minutes to low oxygen pressures; seven exposures were made on alternate days. There was after each exposure a transient rise in blood-agglutinins, and compared with control animals, the exposed animals maintained a higher titre of agglutinins both during and after the exposures. Monaco suggests that agglutinins were mobilized in the blood by the stimulus—oxygen lack—which mobilized the erythrocytes. The effect is clearly analogous to that of bleeding.

It may be accepted that bleeding does act as a stimulus to antibody production in the same sense that it acts as a stimulus to the production of the serum proteins in general; that is, there is an active response tending to make good the loss sustained. Whether this particular stimulus suffices to force the titre, transitorily or for longer periods, far above the pre-existing level is more doubtful.

Another procedure that has been stated to supply a non-specific stimulus to the antibody-forming apparatus is the injection of various metallic salts, colloidal solutions or other substances. We may ignore, from our present point of view, those instances in which the effect of such injections is limited to an increase in the bactericidal power of the blood. Such effects will be more conveniently considered in Chapter 52.

Wallum and his collaborators (Walbum and Mørch 1923, Walbum and Berthelsen 1925, Walbum 1925, 1926, Schmidt 1926, Ørskov and Schmidt 1928) have recorded

numerous experiments along these lines. They conclude that the injection of salts of certain metals, especially those of cobalt, manganese and beryllium, causes an increase in the production of antitoxin, or of agglutinins, if given early in the course of immunization, and leads to a secondary rise in titre, if injected when the antibody titre has commenced to fall. Several investigators have, however, failed to confirm these observations (McIntosh and Kingsbury 1924, Horgan 1925), and the protocols included in some of Walbum's reports suggest that the response is very irregular.

Steabben (1925) studied the effect of various colloids on antibody production. Using agar, gelatin, a silver sol and an iron sol, she found that the injection of these colloids had no power to increase the antibody titre when this had become steady, though the injection of such a colloid at the same time as the bacterial antigen might increase the antibody response. This last effect is clearly analogous to the adjuvant action of certain substances on the response to an antigen, already discussed on p. 1115.

Obermayer and Pick (1904) recorded that animals immunized 3 months previously showed a fresh formation of precipitin after the injection of a 5–10 per cent. solution of peptone. Fleckseder (1916) stated that the injection into human beings of deuteroalbumose or nucleic acid gave rise to an increase in the agglutinins for typhoid bacilli which were already present in detectable amount in the subjects in question. Weichardt and Schräder (1919) noted a definite increase in agglutinin titre after the injection of deuteroalbumose, sodium nucleinate or milk. Matsuda (1924) reported that the injection of deuteroalbumose was followed by a secondary increase in the disappearing viricidal antibodies in the blood of vaccinia-immune animals. Wahl (1938) records a similar effect in rabbits immunized against *Salm. typhi* and *V. cholerœ*. Weichardt (1922) and Wahl both review the data with regard to the effect of protein substances as non-specific stimuli; in most cases the stimulus is irregular, and the antibody response is small compared with that to a specific antigenic stimulus.

Finally we may consider those cases in which the non-specific stimulus is provided by a complex antigen, similar in type to that providing the initial specific stimulus but possessing no known common antigenic factor. So far as this antigenic dissimilarity is in fact complete the case does not differ in its essentials from those we have considered above.

Dreyer and Walker (1909) recorded a secondary rise in agglutinins for *Bact. coli*, following the intraperitoneal injection of killed staphylococci into previously immunized rabbits. Conradi and Bieling (1916) state that rabbits immunized against *Salm. typhi* show a secondary rise in agglutinins after various non-specific stimuli, such as those provided by the injection of *Bact. coli*, dysentery bacilli and *C. diphtheriœ*. Tsukahara (1921) records a series of experiments which are highly suggestive. He injected rabbits with typhoid, paratyphoid and dysentery bacilli, using one organism as the primary specific stimulus and another as the secondary non-specific stimulus, and noted many instances of a secondary rise in agglutinins. A careful perusal of his protocols, however, indicates very clearly that, where the secondary response was well marked, there was some antigenic relationship between the two kinds of bacteria employed, indicated by the fact that a primary inoculation with one of them was followed by a slight but definite production of antibodies acting on the other. This fact, of course, excludes these particular examples from the category which we are considering.

Rosher (1924) was unable to detect non-specific stimulation of antibody production in any of the following instances: primary specific antigen *Bact. coli*, secondary non-specific inoculum *Staph. aureus*; primary specific antigen *Bact. coli*, secondary non-specific inoculum sheep's red cells; primary specific antigen *Salm. paratyphi A*, secondary non-specific inoculum suspension of mixed unrelated bacteria; primary specific antigen *Salm. typhi*, secondary non-specific inoculum *Salm. paratyphi B*; primary specific antigen *Salm. enteritidis*, secondary non-specific stimulus infection with living tubercle bacilli.

In all cases in which this point was examined, the secondary inoculum acted as an effective primary specific stimulus, inducing the production to a relatively high titre of antibodies reacting with its own antigenic components, while leaving the titre of antibodies acting on the previously injected antigen unaltered. Analogous results had been noted by many of the observers referred to above.

To summarize, we may say that there is some evidence that non-specific stimuli —extensive bleeding, or the injection of metallic salts or colloids or protein solutions or unrelated bacteria—may induce (*a*) an increase in the titre of a normal antibody, (*b*) an increase in the production of immune antibodies when administered during the early stages of immunization, or (*c*) a secondary rise in titre when administered after the effect of the preliminary specific immunization has reached its maximum, or declined from it. The effects summarized under (*b*) may well be due to adjuvant effects such as we have discussed above. Wahl (1938) indeed attributes them to the induction of an inflammatory lesion which absorbs and retains antigen still present in the body. The responses falling within these categories are, however, irregular, usually trivial, and never comparable to those which follow the reinjection of the specific antigen. It is quite certain also that the injection of a given antigen may stimulate the antibody-forming apparatus to the energetic production of the corresponding antibody, without any detectable production or mobilization of antibodies acting on an unrelated antigen to which it had previously responded.

The Fate of Antibodies when injected into a Normal or Immunized Animal.

This is a convenient place in which to note a series of observations which, while not immediately related to the phenomena that we have been discussing, are of considerable practical importance.

When we inject an antibody-containing serum into a normal animal, the rate at which it reaches the blood stream and the maximum concentration which it there attains are in great part determined by the route of injection. Obviously we shall obtain the most rapid effect by injecting our antiserum intravenously. Injection by any other route will involve a lag period before the maximal concentration is reached in the circulating blood; and since the antibody-containing serum is being slowly eliminated throughout the whole period the concentration in the blood and tissue fluids in general will reach a lower level.

Smith (1907) records a series of experiments that serve to illustrate these points.

Fig. 262 shows the disappearance of agglutinins for *Bact. coli* from the blood of a rabbit after an intravenous injection of the serum of an immunized goat.

Fig. 263 shows the effect of inoculating the same serum subcutaneously. It will be noted that the maximal concentration of agglutinin in the circulating blood is not reached until 53 hours after the injection.

Fig. 264 shows the concentration of antitoxin in the circulating blood of a human subject at varying intervals after the subcutaneous injection of an antitoxic serum. It will be noted that the peak is not reached until the 3rd or 4th day.

The intravenous injection of antiserum is not necessarily followed by an immediate establishment of a maximum antibody level in the blood. In man, Finland and Brown (1938) noted that maximum titres were not reached until 5 and sometimes 10 hours after intravenous injection. This phenomenon is not readily explained, except in terms of a rapid absorption of antibody to the tissues, and a subsequent shift in the blood-tissue antibody equilibrium, during which antibody is released into the blood. The studies of Fink *et al.* (1944) on the metabolism of plasma proteins in the dog provide some support for the explanation, for it appears that the plasma proteins are normally in constant and

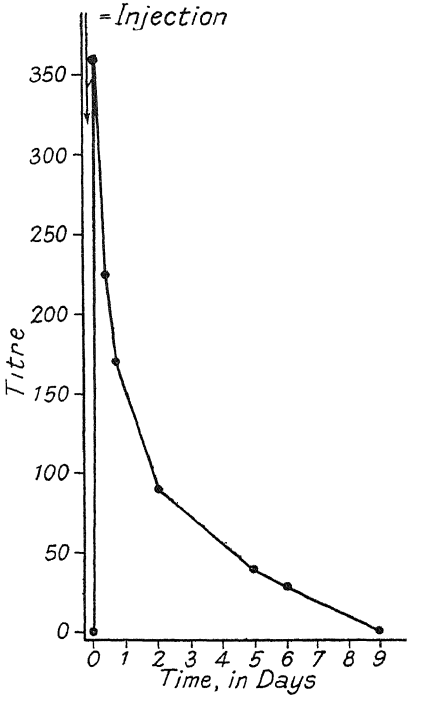


FIG. 262.

Disappearance of agglutinins from circulation of a rabbit after intravenous injection of agglutinating serum. (After Smith.)

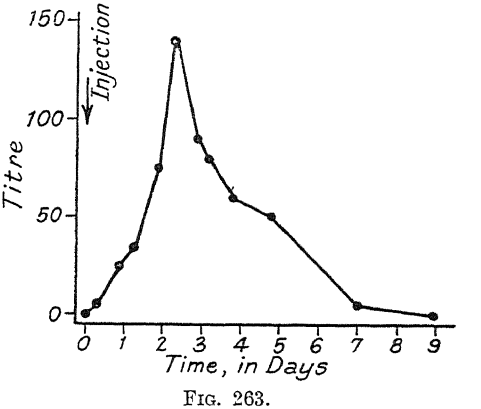


FIG. 263.

Rise and fall of agglutinins in blood of a rabbit after subcutaneous injection of agglutinating serum.

(After Smith.)

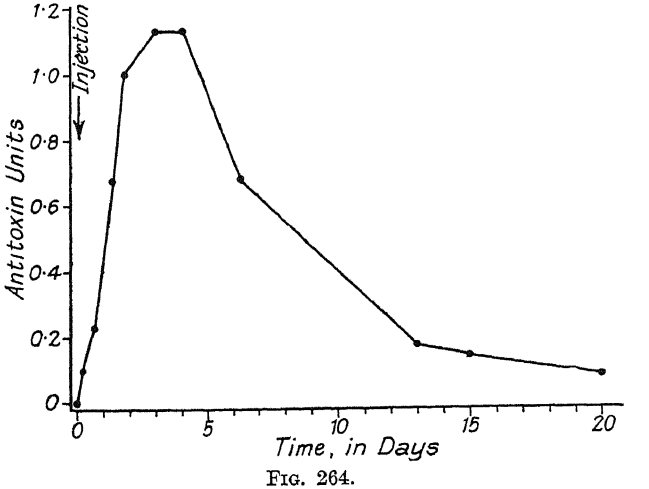


FIG. 264.

Rise and fall of antitoxin in blood of human subject after subcutaneous injection of antitoxic serum.

(After Smith.)

rapid exchange with a readily mobilizable pool of body protein. It is not impossible that injected antibodies may participate in this exchange. In this connection we may note that a large proportion of intravenously injected antibody may disappear from the circulation in a relatively short time. For example, Sédallian, Jourdan and Clavel (1937) replaced the blood of normal rabbits with that from actively immune rabbits, and found that within 10 minutes the antibody titre was one-tenth that to be expected on the basis of dilution of the donor blood in the blood of the recipient animal.

It may be noted that an antiserum injected into the peritoneum rapidly reaches the blood stream *via* the thoracic duct, so that the effect does not differ greatly from that of an intravenous injection. After an intramuscular injection an anti-

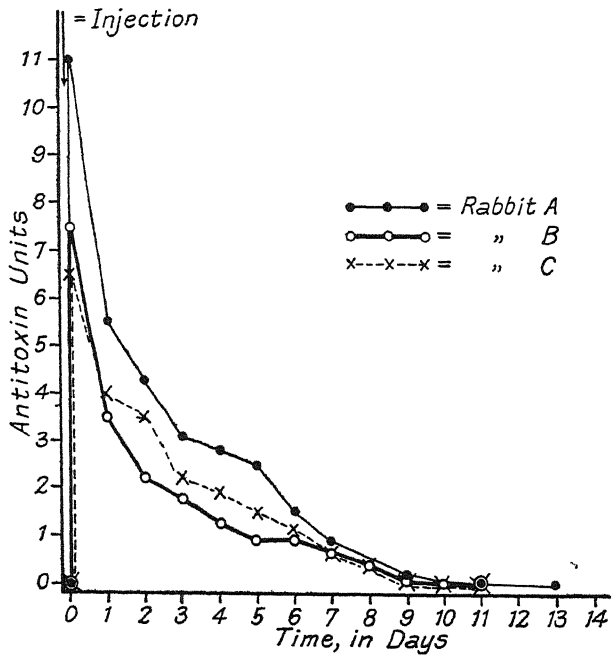


FIG. 265.

Disappearance of antitoxin from blood of three normal rabbits, after intravenous injection of antitoxic horse serum.

(After Glenny and Hopkins.)

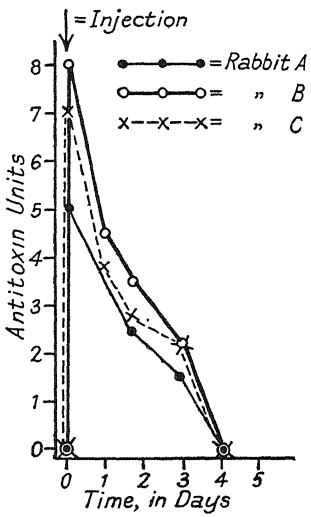


FIG. 266.

Disappearance of antitoxin from blood of three rabbits, previously sensitized to normal horse serum, after intravenous injection of antitoxic horse serum.

(After Glenny and Hopkins.)

serum reaches the blood stream more slowly than after an intraperitoneal injection but more rapidly than after a subcutaneous injection.

Another point of some importance in connection with passive immunization is illustrated in Figs. 265 and 266 (Glenny and Hopkins 1922).

In Fig. 265 is recorded the rate of disappearance of antitoxin from the circulation of 3 normal rabbits after the intravenous injection of 0·5 ml. of diphtheria antitoxin, derived from the horse. Fig. 266 gives a similar record in the case of 3 rabbits that had received previous inoculations of horse serum. These were "immune" or "sensitized" to horse serum—the relation of the immune to the sensitized state is discussed in Chapter 51—and in consequence eliminated the antibody from their circulation much more rapidly than did the normal rabbits.

The same phenomenon will occur when a person who has previously received an injection of horse serum is given an antitoxic or antibacterial serum derived from that animal (see Hooker and Follensby 1931).

The influence of previous sensitization may be even more marked when an antiserum is injected by the subcutaneous or intramuscular route. The cells of a sensitized or immunized animal take up, or fix, some of the antibody distributed throughout the tissues *via* the circulating blood. As a result these cells, or some of them, are able to combine specifically with the corresponding antigen. The intracutaneous, subcutaneous or intramuscular injection of an antiserum prepared in the horse into an animal already sensitized to horse serum, appears to be followed by the fixation of a large proportion of the serum at, or in the neighbourhood of, the site of injection, with a consequent reduction in its therapeutic or prophylactic effect (see Kahn and McDermott 1934, Kahn 1934, Schmid 1938*b*). The fixation is not necessarily due to an absorption of the injected serum to antibody specific for it in the tissues. The inflammatory lesions that follow the injection into the sensitized tissues may also hold the serum in a non-specific manner. Finland and Brown (1938) record that the absorption of antipneumococcal sera was slowest with sera that induced the more severe local reactions upon intramuscular injection.

The Physiological Fate of Antibodies.—The disappearance of passively administered antibody from the circulation within a few weeks suggests that they are rapidly metabolized by the tissues. Normal plasma proteins, including globulins, are utilized by the body as food-stuff (see, for example, Holman *et al.* 1934) and it is reasonable to assume that antibodies are similarly susceptible to tissue enzymes. In this case the antibody levels observed in the blood of actively immunized animals must be maintained by an excess of production over destruction, a destruction which presumably is as rapid as that of passively injected antibodies. Heidelberger, Schoenheimer and their colleagues (Heidelberger *et al.* 1942, Schoenheimer *et al.* 1942) using dietary amino-acids marked with isotopic nitrogen (N_{15}) have shown that the dietary amino-acids are incorporated into the plasma proteins, including the antibodies, of an actively immunized animal, but not into passively injected antibodies. The rate of appearance and disappearance of the marked nitrogen from the blood stream indicated that antibody was formed rapidly from the dietary amino-acids, and had a " half-life " of about 2 weeks, a finding which accords well with the inferences drawn from the data for the disappearance of passively administered antibody.

The Mechanism of Antibody Formation.

As to how antibodies are formed, with the single exception of Pauling and Campbell's (1942) work, we have no positive knowledge that is based on direct experiment. In the early days of immunology the hypothesis that the injected antigen was in some way incorporated in the antibody was tentatively advanced. It had the obvious merit of offering a possible explanation of specificity, but was soon abandoned because of the enormous quantitative disparity between the antigen injected and the antibody formed. More recently it has been revived in a less direct form ; but it is difficult to accept in any form at all.

In certain of the experiments recorded above it was calculated that the dose of active antigenic material injected into a 2,000 gm. rabbit was of the order of 0·000,000,5–0·000,005 mgm., yet this dose might result in the production of agglutinin to a titre of 1 : 500. Hooker and Boyd (1931) have calculated that, taking such relationships into account, one molecule of active antigenic substance must give rise to an amount of antibody globulin sufficient to agglutinate 600 bacteria ; and, since the surface relationship between one molecule of globulin and 600 bacteria is of the order of 1 : 25,000,000, there seems no room for any hypothesis which assumes the permanent incorporation of any fraction of the antigen in the antibody complex. Berger and Erlenmeyer (1931) and Hooker and Boyd (1931, 1932) have examined

this question experimentally, using a linked synthetic antigen containing arsenic, and testing for the presence of arsenic in the antibody-containing serum. These tests were entirely negative and the limits thus set to the amount of antigen present in the antiserum appear to render it impossible that a molecule of antibody can contain any grouping derived from the antigen. If the antigen is incorporated in the antibody at any stage in the formation of the latter, it must apparently be released again, perhaps leaving its impress on the complex of which it once formed a part, and by repeated reassimilation modifying successive fractions of the serum globulin (see Topley 1930).

A working hypothesis of the method of antibody formation, that has a reasonable *a priori* probability and accords well with our present knowledge of the structure of antigens and antibodies, has been put forward independently by Breinl and Haurowitz (1930), Alexander (1931) and Mudd (1932). Ignoring minor differences in the views of its sponsors, this hypothesis assumes that all antibodies are globulins newly synthesized under the directing influence of the antigen. This influence is exerted through a union, physical or chemical, between the peptides or amino-acids and the active groupings of the antigen, during the globulin synthesis. Mudd lays special stress on the point that this synthesis occurs at the interface between the antigen and the surrounding fluid, a point of particular interest in view of the observation that the antibody content of an antibacterial serum seems to be largely determined by the nature of the active groupings at the cell surface. The globulin thus formed is assumed to be dissociated from the antigen after a certain stage has been reached, and the latter is thus able to leave its stereochemical imprint on successive molecules of antibody globulin, synthesized under the same conditions. Breinl and Haurowitz would locate this synthesis in the reticulo-endothelial cells, the antibody globulin being released when these cells disrupt; or, as Sabin (1939) has suggested, both normal and antibody globulin may be shed into the blood stream from the outer layer of the reticulo-endothelial cell. Pauling (1940) has reviewed the physico-chemical bases of the hypothesis, and considers that the difference between normal and antibody globulin lies not in the ordering of the amino-acid residues of the polypeptide chains, but in the manner of the folding of their otherwise similar constituent polypeptide chains, to form polypeptide laminæ and ultimately the globular protein. According to Pauling, the ends of the folds, which when packed together will constitute one of the surfaces of the completed molecule, are capable of taking up many configurations, whose variety and stability are determined by the presence of an excess of proline and hydroxyproline residues at the point of folding. When an antigenic substance with a well-defined configuration of active groups is present in the cell, it influences the configuration at the ends of the folded polypeptide chains, thereby inducing the formation of an antibody receptor. Pauling suggested that if globulin molecules were unfolded by denaturing agents and allowed to re-fold in the presence of an antigen or hapten, antibody might be formed.

Pauling and Campbell (1942) reported the *in vitro* synthesis of antibody-like protein by this means, thus providing strong support for their hypothesis. When ox γ-globulin was partly denatured by exposure to 57° C. for 14 days, in the presence of the azo-dye dihydroxy-tri (*p*-azophenylarsonic acid) benzene, a precipitate of dye and protein was formed, presumably consisting of modified globulin united specifically to the " antigen." The dye was then displaced from the insoluble complex by dialysis against a solution of its " determinant " group, namely sodium *p*-amino-phenylarsonate, and the arsonate then removed by dialysis against NaCl. About 15 per cent. of the protein in the resulting solution had certain antibody properties; it was specifically precipitated by the azo-dye,

and precipitation was specifically inhibited by the arsonate. A similar specific modification of ox-globulin was produced, using the polysaccharide of the Type III pneumococcus as the "antigen."

Burnet (1941) has pointed out a number of features of antibody-formation that do not accord well with the "scaffold" hypothesis. In the first place the hypothesis demands the presence of antigen in the tissues as long as active immunity lasts. Immunity to smallpox, for example, may last 20 years (see Chapter 86). Are we, therefore, to conclude that the smallpox antigens remain in the tissues for 20 years? We can clearly postulate a persistence or a slow multiplication of virus in the body over this period, or the maintenance of immunity by continued reinfection (see Chapter 55). At present, however, the evidence for this view is purely question-begging, in that it is based on observations of long-lasting immunity. As regards the persistence of inanimate antigen, we have little relevant information. Antigenic material like bacteria and red cells rapidly disappears from the reticulo-endothelial cells, but we have no basis for supposing that their constituent parts disappear with equal rapidity, and it is not necessarily absurd to assume that long-lasting immunity is equivalent to a long-persisting antigen. But even so, we shall have to decide where it persists. Returning to our smallpox example, it is reasonable to suppose that in 20 years the reticulo-endothelial cells of the tissues will be the remote descendants of those that originally ingested the antigen, in which case the persisting antigenic units must pass to one of the daughter cells at each division. Burnet prefers the assumption of an antibody-forming capacity, independent of the continued presence of antigen, which is transmitted to descendant cells by some hereditary process.

He postulates that the serum globulins are products of self-reproducing, intracellular, globulin proteinases, and represent a stage in synthesis when the molecule has the essential structure of the enzyme, but is without enzymic activity. At this stage it may be turned out into the circulation as a globulin. In the cell, however, the synthesis proceeds to completion, and more proteinase is formed. In the presence of antigen, the configuration of the proteinase is modified so as to hydrolyse the antigenic matter, and, when the antigen is gone, the proteinase reproduces itself in the modified form, both in the original cell and its descendants. According to Burnet, the continuously accelerated output of antibody observed during the secondary response is characteristic of an autocatalytic process such as he postulates, not the production of antibody from a fixed number of antigenic scaffolds; and he attributes the change in reactivity of antibody observed with prolonged immunization (see Chapter 7) to the profounder modification of the globulin proteinases by the products of a more complete intracellular hydrolysis of the antigen.

We are not in a position to decide between these hypotheses. Both depend upon arguments by analogy with other biological processes, and it is not in all cases clear how relevant these analogies are. The student is referred to Pauling (1940) and Burnet (1941) for a fuller discussion. The evidence is also reviewed by Sevag (1945), who develops the conception of antigens as specific catalysts of antibody formation, basing his argument on a number of analogies between the properties of antigens and enzymes. This, it may be noted, is in direct contrast to the views of Burnet, who regards antigens as modifiers of the intracellular proteinases.

SUMMARY

(1) The available evidence indicates that the cells of the reticulo-endothelial system, which are known to remove injected antigenic material from the blood and tissue fluids, play an essential part in the production of antibodies. Whether these cells themselves produce the antibodies in their final and complete form, or whether their rôle is confined to the temporary retention of antigen and its subsequent liberation in an altered or unaltered state, the actual synthesis of antibodies occurring elsewhere, cannot yet be determined. The present indications are that the part played by the reticulo-endothelial system is not confined to the retention and release of unaltered antigens. There is also evidence that lymphoid tissue is concerned in the production of antibodies.

(2) The antibody-forming apparatus responds in a characteristic way to a specific stimulus provided by the introduction of an antigen into the tissues. This response differs as regards its time relations, extent and even its occurrence or non-occurrence, in different animals and with different antigens, but its general form is of a well-defined type. Following an optimal injection of an antigen which evokes an effective primary response there is a lag period of a few days followed by the appearance in the blood of the corresponding antibody. The titre of this antibody rises rapidly to a maximum which is usually attained between the 10th and 22nd day after inoculation. It then falls, at first rapidly then more slowly, reaching a level which is maintained with little alteration for several months. The final disappearance of antibodies from the blood stream may not occur for many months, or even years.

(3) The amount of antibody produced in response to a single injection of antigen varies with the amount of antigen injected. There is a threshold dose—often extremely small—below which no response is obtained. With increasing doses there is an increase in antibody production as judged by the maximal titre attained in the blood, but the increase is not in simple proportion; a large increase in dosage usually results in a relatively smaller increase in titre. As the dose increases still further the ratio of increase in titre to increase in dose grows smaller and smaller, until a maximum effective dose appears to be attained, provided that a lower limit of dosage is not imposed by the toxicity of the antigenic material.

(4) The antibody-forming apparatus is capable of responding simultaneously to the injection of several different antigens, but with more than two or three antigens the multiple response tends to be less effective against any one antigen than if it were responding to that antigen alone.

(5) The antibody-forming apparatus displays a characteristic difference in its response to primary and subsequent injections of the same antigen. Following a secondary stimulus antibody production is usually more rapid and more copious than after the primary stimulus, and the decline in titre of circulating antibodies after a maximum has been reached is usually delayed. An immunized differs from a normal animal, not only in the possession of a particular antibody, but in its capacity to produce that antibody rapidly and in large amount in response to the specific stimulus.

(6) Repeated inoculations are on this account more effective in raising the antibody titre to a high level than a single injection, even of a very large dose. Just as there appears to be a limit to the titre that can be attained by increasing

the size of a single dose of antigenic material so there is a limit beyond which repeated injections fail to force the titre.

(7) The injection of a large dose of antigen into an animal in whose blood the corresponding antibody is present frequently results in a temporary fall in the concentration of that antibody, followed in most cases by a secondary rise.

(8) Antibody formation may be induced by the administration of antigens by the mouth, by inhalation, or by intranasal instillation. Administration by these routes is, in general, less effective than direct inoculation into the tissues. In the case of administration *per os* there is evidence that antibodies may be produced against certain antigenic components but not against others.

(9) Although the majority of naturally occurring antigens are proteins, or contain protein components, recent observations have shown that other substances, such as complex polysaccharides, are capable of inducing an active immunity, and of stimulating antibody production.

(10) The antibody-producing apparatus may in certain conditions be stimulated by non-specific substances. Two effects may be distinguished:

(*a*) An adjuvant action of substances injected into the tissues simultaneously with antigen, or during the period of increasing antibody response. Adjuvants appear to act mainly by inducing tissue lesions from which the absorption of antigen, and its consequent elimination, is retarded. A stimulus to the multiplication of antibody-forming cells cannot be excluded from the adjuvant effect.

(*b*) A stimulation of the antibody-forming apparatus that results in some increase in the concentration of a normal antibody or in the renewed production or mobilization of an antibody previously produced in response to a specific stimulus. The evidence for such stimulation is conflicting; it is clear that this non-specific response occurs very irregularly, is usually trivial or transitory in its effect, and is never comparable to the response to a further injection of the specific antigen.

(11) The rate at which antibody, contained in any foreign antiserum, reaches the blood and tissue fluids of a normal animal and the concentration there attained depend in great part on the route by which the antiserum is injected. If the animal or man has received a previous injection of any serum of the same animal origin the antibody will be eliminated at a greatly increased rate.

(12) The mechanism of antibody formation still awaits elucidation. Various hypotheses have been advanced, but none of them completely covers the observed phenomena. The most generally accepted working hypothesis—that the configuration of the globulins is modified during synthesis in the presence of antigen so that the surface of the completed molecule is complementary to the antigenic surface—has the support of a recent demonstration of the *in vitro* modification of globulins in the presence of antigens, with the production of proteins having some of the properties of antibody produced *in vivo*.

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CHAPTER 51

ANAPHYLAXIS, HYPERSENSITIVENESS AND ALLERGY

In the preceding chapters we have considered a series of reactions most of which have as a common factor the property of affording specific protection against bacteria or their products. Numerous observations, both clinical and experimental, have afforded instances of the contrary condition, in which an individual is, or may be rendered, more susceptible to some noxious agent than are the generality of his own species. In some cases an individual may become highly sensitive to an agent which is entirely innocuous to his normal fellows. The phenomenon of hypersensitiveness is presented in its clearest and least ambiguous form when the exciting agent is some non-living material, so that there is no question of multiplication within the body or of the complex and variable tissue reactions that are associated with bacterial infection. It will be convenient to consider first the data relating to this type of abnormal sensitivity, and then to discuss its bearing on the phenomena of bacterial disease. It will also simplify matters to start with the most striking and clear-cut case of hypersensitiveness—the phenomenon of acute anaphylactic shock.

ANAPHYLACTIC SHOCK

The detailed study of this curious reaction dates from the beginning of the present century, though several observations are on record in the earlier literature which with our fuller knowledge we can recognize as undoubted examples of anaphylaxis. The observations of Richet and his co-workers (Portier and Richet 1902, Richet 1907) first drew general attention to the experimental facts; though the issue was at this stage obscured by the chance that the agent selected for study was itself toxic for the experimental animal employed. Richet observed that dogs which had survived a single injection of an extract of certain actinians might succumb rapidly, and with a curious and characteristic symptom complex, to a second injection of the same material given after days or weeks. It was this substitution of increased susceptibility for the increased resistance which usually follows the injection of sublethal doses of a toxic material that led him to coin the term *anaphylaxis*.

At about the same time Theobald Smith in America had noted the abnormal sensitiveness of guinea-pigs to widely spaced injections of toxin-antitoxin mixtures. He communicated these observations to Ehrlich in 1904; and Otto in 1905 published the results obtained in a detailed study of this phenomenon. He showed that the horse serum in the mixtures, not the toxin, was the essential agent; that an interval of 10 days elapsed after the sensitizing dose before hypersensitiveness

was established; and that no hypersensitiveness resulted when large injections of serum were given at short intervals.

The same phenomenon was studied independently by Rosenau and Anderson (1906, 1907), who established the strict specificity of the reaction. They found that guinea-pigs sensitized to horse serum showed little if any hypersensitiveness to the serum of other animals, such as the rabbit, cat, dog, pig, sheep, chicken or man. Similarly, guinea-pigs sensitized to horse serum did not respond in any abnormal way to the subsequent injection of egg-white, vegetable protein or milk. They noted also that the amount of serum required to produce sensitization was extremely small; as little as 0·000,001 ml. sometimes induced typical hypersensitiveness. The amount required to produce anaphylactic shock in a sensitized animal was, however, considerably greater (0·01–0·1 ml.). They confirmed the existence of a latent period of 10–12 days between the sensitizing injection and the establishment of the anaphylactic state, and noted that, once established, this state lasted for an indefinite period, extending over several months at least.

These observations have been repeatedly and consistently confirmed by later workers. Animals may be actively sensitized by the injection of any substance that is antigenic in the full sense. The sensitizing injection may be given by any route. Often a single sensitizing dose suffices. In some animals, and with some antigens, it is necessary to give more than one sensitizing injection, and to use doses far larger than the minute amount that suffices in the particular case of the sensitization of the guinea-pig to horse serum; but the same principle holds—the injections must not be too large, nor too often repeated.

As regards the shock-inducing, or "shocking" injection, either the antigen used for sensitization, or certain types of separated haptens containing the specific reactive grouping, are effective in eliciting the characteristic symptoms. The route of injection is important. Intravenous inoculation gives the most constant results; in some animals it is the only effective route for this purpose. In the guinea-pig, which is peculiarly susceptible in this respect, shock may be produced by intraperitoneal inoculation, but larger doses are required. Within certain limits the size of the shocking dose, nevertheless, appears to be a secondary factor in determining the severity of shock. In dogs, for example, Dragstedt (1943*b*) found that shock did not become more fatal with increase in size of the shocking doses; the fatality was primarily determined by the degree to which the animal had reacted to the sensitizing dose.

A curious and significant fact emerged early in the widespread investigation which followed the opening-up of this new field of research. The symptoms and lesions of anaphylactic shock were found to be constant for a given animal species, irrespective of the nature of the sensitizing antigen; while these symptoms and lesions differed sharply from one animal species to another. Thus the guinea-pig reacts in the same way when anaphylactic shock is produced with horse serum, egg albumin, milk or various bacterial antigens; but the reactions of the sensitized guinea-pig, rabbit and dog to horse serum differ sharply and characteristically from one another.

Anaphylactic Shock in the Guinea-pig.—After a sensitized guinea-pig has received an intravenous injection of the specific antigen, it at first remains quiet; but within a minute marked restlessness develops. The hair begins to bristle over the head and neck, and the animal usually rises on its hind legs, rubs its nose vigorously, and emits loud spasmodic sneezes. Often it gives a jump, or a series of jumps. Within a few minutes tonic and clonic convulsions set in, and the animal falls on its side. The respiration

becomes slow, the mouth opening with each violent inspiratory effort; and breathing ceases within a few minutes. The heart continues to beat for several minutes after breathing has ceased.

The post-mortem findings were first described in any detail by Gay and Southard (1908); but it is to Auer and Lewis (1910) that we owe the first adequate account of the physiological reactions that lead up to the fatal issue. In guinea-pigs examined immediately after death from fatal anaphylactic shock, these observers confirmed the presence of intestinal congestion and the occurrence of active peristaltic movements that had been previously noted. They observed the frequency of hæmorrhages on the under side of the diaphragm, and noted, as Gay and Southard had done, the distension of the lungs; but they showed that this distension was not due to simple emphysema. The lungs remained distended long after the thorax was freely opened. Even when removed from the body they showed no sign of collapse; and portions cut off from the pulmonary lobes, if not too small, remained in their condition of dilatation. More detailed examination showed that the distension was caused by contraction of the bronchioles, which retained the inspired air in the dilated alveoli. The death of the animal was clearly referable to suffocation consequent on this obstruction to respiration. Auer and Lewis showed that the bronchiolar contraction was not prevented by section of the vagi or by the administration of curare, and was therefore of muscular or immediately neuro-muscular origin. They showed also that the blood pressure during anaphylactic shock at first rose considerably and then fell slowly but steadily till death, as one would expect in asphyxia.

By varying the mode of administration of the shocking dose, a delayed fatal shock may be produced in guinea-pigs, in which the pulmonary emphysema is absent, and the gross changes in the organs are confined mainly to intestinal congestion and hæmorrhage (Williamson 1936).

Anaphylactic Shock in the Rabbit.—The rabbit, like the guinea-pig, suffers from acute anaphylactic shock if the sensitization is adequate, and if a sufficient amount of antigen is subsequently administered by the intravenous route. The reaction is not, however, so readily elicited. The symptoms of shock differ in several important respects. The preliminary signs of irritation, the coughing and the violent inspiratory efforts are absent. The animal lies with outstretched legs or falls on its side, gives a series of convulsive movements often associated with the passage of urine and fæces, and dies. In many cases irregular respiratory movements continue for a brief period after the heart has ceased to beat—a reversal of the order of cessation observed in the guinea-pig. At necropsy the pulmonary dilatation, which is so striking a feature in the guinea-pig, is absent in the rabbit. The most characteristic abnormality found in the latter is the extreme dilatation of the right side of the heart. Coca (1919) showed that this dilatation was associated with marked obstruction to the pulmonary circulation, evidenced by the increased pressure required to drive fluid from the pulmonary artery to the left auricle. He concluded that acute anaphylactic death in the rabbit was due to a spasmodic constriction of the branches of the pulmonary artery, followed by rapid dilatation of the right side of the heart and acute heart failure. His observations and conclusions have been confirmed by other workers (Drinker and Bronfenbrenner 1924, Grove 1932*a*, *b*).

Anaphylactic Shock in the Dog.—A sensitized dog, after receiving an intravenous injection of the specific antigen, shows marked signs of restlessness and excitement. Within a few minutes the animal vomits and usually passes urine and fæces, the latter sometimes blood-stained. It then collapses and lies prone. The muscles of the limbs are relaxed. and the dog appears extremely weak. Respiration is slow, and often deep and laboured, Stridor may be heard, and a little froth may exude from the lips. In rapidly fatal shock the weakness progresses, renewed diarrhœa and vomiting set in, and coma, occasionally associated with epileptiform convulsions, leads on to death. It has been pointed out by Dean and Webb (1924) that the reaction almost always occurs in two stages, the initial acute symptoms being followed by some signs of recovery. In fatal cases these soon give

place to the second stage of increasing collapse. In other instances severe initial shock is followed by a rapid return to normal.

As pointed out by Biedl and Kraus (1909), anaphylactic shock in the dog is associated with a progressive fall in blood pressure; later investigations (Pearce and Eisenbrey 1910, Dale 1920*b*) have made it clear that this is not due to any failure of output from the heart or to any influence of the central nervous system, but to the collection and stagnation of the blood somewhere in the tissues. The studies of Dale and his colleagues (Dale 1920*b*) render it highly probable that capillary dilatation is the essential factor.

Manwaring (1910) performed a large number of experiments in an attempt to localize the site of reaction; and, having found that shock did not occur when the abdominal vessels were ligated, proceeded to explore the rôle of the various abdominal viscera by a series of operative experiments designed to exclude each in turn. He found that the exclusion of the liver inhibited acute shock in dogs; and his observations have been amply confirmed by subsequent workers (Voegtlin and Bernheim 1911, Denecke 1914). These results accord well with the findings at necropsy. The congestion of the abdominal viscera has been noted by many observers; and Weil (1917) points out that in dogs dying of anaphylactic shock in its acutest form the single outstanding feature at necropsy is the enormous distension and congestion of the liver.

We may briefly note certain additional phenomena, which seem to be general to anaphylactic shock as displayed by any of the species so far investigated, though not developed to an equal degree in all of them. There is commonly a marked leucopenia; and this, as Webb (1924) has shown, is associated with an aggregation of polymorphonuclear cells in the capillaries of the lungs, a phenomenon clearly analogous to that which follows the intravenous injection of bacteria in immunized animals. In rabbits, the arterioles of the ear are conspicuously contracted during shock; circulating leucocytes adhere to the capillary walls and in some cases form leucocytic emboli (Abell and Schenk 1938). Removal of circulating leucocytes and their retention in the tissues has also been demonstrated by Dragstedt, Ramirez de Arellano and Lawton (1940) in the isolated lung of sensitized rabbits, when perfused with blood containing the specific antigen. There is a decrease in the coagulability of the blood, most marked in the dog. The work of a number of investigators (see Dragstedt 1941) has excluded changes in the blood elements concerned in normal coagulation as the cause of this phenomenon, which appears to be due to the liberation of heparin during shock (Jaques and Waters 1940). There appears to be a decrease in the amount of complement in the circulating blood; and there is a marked fall in body temperature.

Anaphylactic Shock in Other Animals.—Although the guinea-pig, rabbit and dog have been studied more extensively than any other species in regard to their anaphylactic reactions, a considerable number of experiments have been carried out on other animals; and it is instructive to note that, in addition to striking differences in behaviour and physiological reaction during anaphylactic shock, various species differ widely from one another in the ease with which they can be sensitized. Thus, among rodents, the rat appears to occupy a peculiar position in that it is extremely refractory to sensitization (Longcope 1922, Parker and Parker 1924, Spain and Grove 1925); though an anaphylactic response can be demonstrated under suitable conditions (Kellaway 1930). Mice are also resistant, though Weiser and his colleagues (1941) point out that the refractoriness varies with the breed of animal, and in some cases depends on insusceptibility to sensitization, not to insusceptibility to shock. In both the rat and the mouse adrenalectomy increases the susceptibility to shock. Birds, so far as they have been examined, may be sensitized by the usual procedures (Friedberger and Hartoch 1909); and the anaphylactic reaction in the pigeon has been studied in some detail (Gahringer 1926, Hanzlik and Stockton 1927). Sensitization to foreign proteins, associated with phenomena which are anaphylactic in kind but do not include typical shock, has been described in cold-blooded animals such as the frog (Goodner 1926). In monkeys, and this is of particular interest for the student of human pathology, sensitization appears to be induced irregularly and with considerable

difficulty. Zinsser (1920) reports his failure to sensitize *rhesus* or ringtail monkeys with a single injection of horse serum; and he could produce only mild anaphylactic symptoms after treatment with repeated doses of antigen. Shock is accompanied by a diminution in circulating platelets, and, as in other animals, a profound leucopenia (Kinsell *et al.* 1941).

The problem of human anaphylaxis will be most conveniently considered in relation to the problem of natural hypersensitiveness.

Passive Anaphylaxis.

It was noted by some of the earliest workers in this field that female guinea-pigs which had been actively sensitized gave birth to young that were themselves hypersensitive. Otto (1907) first demonstrated that the anaphylactic state could be passively transferred by injecting the serum from an anaphylactic into a normal animal, and in so doing placed anaphylactic shock in the category of the *in vivo* antigen-antibody reactions. His findings were soon confirmed and extended by other workers, in particular by Doerr and Russ (1909), and the numerous observations which have since been recorded place the following propositions on a firm basis of experimental fact, so far at least as the guinea-pig is concerned. A normal animal that has received an injection of the serum of an actively sensitized animal will respond to the subsequent injection of the corresponding antigen by developing acute anaphylactic shock. Passive sensitization may be effected with equal or even greater certainty by an injection of the serum of an animal that has been "immunized" against a given antigen by repeated injections, so that it is not itself sensitive to the antigen in question. In either case the injection of the serum is not usually followed by the *immediate* sensitization of the recipient. An interval of some hours must elapse between the injection of the sensitizing serum and the injection of the corresponding antigen if typical shock is to be regularly obtained. For maximal sensitization the interval is even longer—4 to 6 days (Kellaway and Cowell 1922).

Under suitable experimental conditions, an analogous reaction, "reversed passive anaphylaxis," may be induced in the guinea-pig by injecting antigen first and antibody later (see Kellett 1935). Both in "reversed" and direct anaphylaxis, shock is sometimes produced when the two intravenous injections follow one another immediately (Dean *et al.* 1936, Zinsser and Enders 1936).

The efficacy of an antiserum in the induction of passive anaphylaxis appears to be closely related to its content in specific antibody as recorded by precipitin tests (Doerr and Russ 1909), and there seems no reason for supposing that the reagents involved differ from those with which our other immunological studies have made us familiar. The experimental study of this phenomenon has, however, brought to light a relationship that is probably of fundamental importance in immunological mechanisms. Different antisera, produced by the immunization, or sensitization, of animals of different species against a single antigen, differ widely in their ability to induce passive sensitization in a given species of animal.

Thus, Avery and Tillett (1929) and Brown (1934*b*) found that guinea-pigs could be sensitized to pneumococcal polysaccharides by the injection of antipneumococcal sera prepared in the rabbit, but not by antipneumococcal sera prepared in the horse. Again (see Friedberger and Hartoch 1909, Scott 1931, Hartley 1940), guinea-pigs cannot be sensitized with the serum of fowls, oxen, goats, sheep, and pigs, and pigeons cannot be sensitized with the serum of rabbits. The mouse would appear to resemble the guinea-

pig, in that it can be passively sensitized with antipneumococcal serum prepared in the rabbit but not with antipneumococcal horse sera (Mehlman and Seegal 1934). Chicken antisera will not sensitize mice (Weiser *et al.* 1941).

The insusceptibility of guinea-pigs to sensitization with equine antibody has been questioned by Bailey, Raffel and Dingle (1937), who reported sensitization by equine antipneumococcal sera, and attributed their success to the injection of large shocking doses of antigen, sufficient to overcome any protection afforded by unfixed antibody (see below) in the circulation. Using equine antisera to hæmocyanin and ovalbumin, Follensby and Hooker (1944) were unable to sensitize guinea-pigs, though the shocking doses of antigen, as in Bailey's experiments, were large, and greatly in excess of the amounts serologically equivalent to the antibody injected (Chapter 7). They suggest that optimal quantitative relations between the antibody and antigen employed cannot wholly explain Bailey's results.

It will be seen in later sections of this chapter that there are reasons for believing that the fixation of the injected antibody by the tissue cells of the recipient is an essential factor in passive anaphylaxis; and it is not surprising to find that the serum globulins of different species behave differently in a reaction of this kind.

The amount of antibody required for passive sensitization has been measured by Kabat and Landow (1942) and Kabat and Boldt (1944). Full sensitization to fatal shock was produced by the injection into guinea-pigs weighing 250 gm., of about 0·03 mgm. of antibody nitrogen; that is, less than 0·2 mgm. of antibody globulin. With this minimal sensitizing dose, the optimal shocking dose of antigen—ovalbumin—was about 50 times the amount reacting optimally in a constant-antibody precipitin titration.

The phenomena of active and passive anaphylaxis appear, as we have seen, to depend on specific reactions of the antigen-antibody type, and there seems no purpose in burdening our vocabulary with additional labels, such as "anaphylactogens" or "anaphylactic antibodies." It is, however, of interest to inquire how partial antigens, or haptens, behave in this reaction.

The Rôle of Haptens in Anaphylaxis.—Tomcsik and Kurotchkin (1928) immunized rabbits against *Bact. aerogenes*, the pneumobacillus, and a yeast. In each case they were able to obtain a high-titre precipitating serum for the polysaccharide hapten extracted from the corresponding organism. Guinea-pigs injected with these rabbit antisera developed acute anaphylactic shock when subsequently injected with the corresponding hapten. Lancefield (1928) sensitized guinea-pigs with the sera of rabbits immunized against hæmolytic streptococci, and induced acute shock by the injection of the separated haptens. Enders (1929) obtained similar results with a polysaccharide fraction from the tubercle bacillus. Avery and Tillett (1929) and Brown (1934*a*) have studied the anaphylactic action of the pneumococcal polysaccharides. These haptens will not induce active sensitization in the guinea-pig, but they will induce acute anaphylactic shock in animals passively sensitized by the injection of precipitating antisera prepared in the rabbit, and these reactions are type-specific. Morgan (1932) records a similar relationship between the specific polysaccharide of *Sh. shigæ* and guinea-pigs passively immunized with the homologous antibacterial serum.

Tillett, Avery and Goebel (1929) have tested the anaphylactic response to synthetic sugar-proteins. They prepared *p*-aminophenol glucoside and *p*-aminophenol galactoside and linked these to serum globulin and to egg albumin. Preliminary precipitation tests showed that antisera produced in rabbits reacted specifically *in vitro* with the carbohydrate groupings. Guinea-pigs passively sensitized by the injection of an anti-glucoside-globulin serum developed typical anaphylactic shock when subsequently injected with glucoside-albumin. Guinea-pigs sensitized with anti-galactoside-globulin serum reacted similarly

when injected with galactoside-albumin. In each case the reaction was shown to be specific. Active sensitization with the globulin compounds, followed after 11 days by injection of the corresponding albumin compounds gave similar results. The unconjugated glucoside or galactoside did not induce shock when injected into sensitized animals, but each inhibited anaphylactic shock when injected immediately prior to the injection of the corresponding carbohydrate-protein compound. Klopstock and Selter (1929) carried out similar experiments and obtained similar results, using compounds of protein with atoxyl, or with the sodium salt of metanilic acid. Landsteiner and Levine (1930) sensitized guinea-pigs to the *d*- and *l*-isomers of *p*-amino-tartranilic acid, in each case coupled to horse globulin. The subsequent injection of the corresponding active groupings coupled to chicken globulin induced anaphylactic shock. The shock could be specifically inhibited by a preliminary injection of a compound of resorcinol with the active grouping concerned. Similar results were obtained with guinea-pigs passively sensitized to *p*-amino-arsanilic acid, the active substance being coupled to tyrosine for the inhibition tests. In later experiments Landsteiner and van der Scheer (1932, 1933, 1938) were able to induce shock by injection of the azo-dye compounds alone into animals sensitized with the appropriate azo-dye-proteins.

Specific Desensitization.—The specific desensitization of sensitized animals was recorded by many of the earlier investigators (Otto 1905, Rosenau and Anderson 1906, 1907, 1908, Besredka and Steinhardt 1907, Gay and Southard 1908). Desensitization—as then observed, and in the sense in which this term is now commonly employed—differs from the inhibitory effect induced by simple haptens in that the desensitizing agent is the complete antigen, which is capable, when administered intravenously in adequate doses, of inducing typical shock. The desensitizing effect is obtained, in place of acute shock, if a very minute dose of antigen is given and repeated, or if rather larger doses are given by a route, such as the subcutaneous, which ensures slow absorption. The essential conditions for success appear to be the administration of an amount of antigen that is adequate in total by some method that prevents any rapid accumulation in the circulating blood. It would seem that desensitization, however it is induced, is purely temporary, the anaphylactic, or hypersensitive state being re-established in a few days or weeks (Valléry-Radot *et al.* 1936, Adelsberger 1936, Gernez 1936).

The Mechanism of Anaphylactic Shock.

It is clear that acute anaphylactic shock, as induced by any of the procedures that have been described above, belongs to the category of the specific antigen-antibody reactions. The only question at issue is the mechanism by which this primary reaction gives rise to the series of events that characterize acute shock in any given animal species.

Two opposing views have been put forward. According to one the reaction between antigen and antibody, occurring in the circulating blood or in the tissue fluids, gives rise to a toxic product, anaphylatoxin, which, acting on susceptible cells, gives rise to the characteristic syndrome. According to the other the primary reaction occurs not in the blood or tissue fluids but on or in the tissue cells; and the reactions that characterize acute shock are due not to any toxicity of the antigen-antibody compound itself, or of any derivative from it, but to cellular disturbances initiated by the antigen-antibody reaction. As we shall see, the picture that has been pieced together during the last few years derives, in all its essential outlines, from the cellular school, though it contains details reminiscent of the anaphylatoxin hypothesis.

The Humoral or Anaphylatoxin Hypothesis.—The chief protagonist of this conception of the mechanism of acute anaphylactic shock was Friedberger (1910, 1912, 1913), who had at an earlier period been one of the sponsors of the cellular hypothesis. Certain difficulties in the latter theory, not easily explicable in the light of the knowledge then available, had caused him to modify his views; but his complete *volte-face* was largely determined by the observation of Friedemann (1909) that a guinea-pig could be poisoned with the characteristic symptoms of acute shock by injecting a mixture of an antigen and its homologous antibody after a brief period of incubation *in vitro*.

The humoral theory in its original form postulated a union of antigen with antibody in the circulating blood, followed by a cleavage of the resulting complex, presumably of the hydrolytic type, and the setting free of toxic degradation products—a conception which seemed to accord well with the findings of Vaughan and Wheeler (1907) on the toxicity of the intermediate products of proteolytic degradation in general. Friedemann's observations, which have been repeatedly confirmed, seemed at first to afford a direct demonstration of the production of anaphylatoxin from antigen and antibody *in vitro*; but it soon became clear that sera could be rendered toxic, in this sense, by a variety of procedures in which antigen and antibody played no part. Thus anaphylatoxin can be produced by incubating normal serum with kaolin (Ritz and Sachs 1911, Keysser and Wassermann 1911, Mutermilch 1913), barium sulphate (Mutermilch), talc (Mutermilch), starch (Ritz and Sachs 1918), inulin (Nathan 1914), or agar (Bordet and Zuntz 1914.)

The humoral theory in its earlier form was often described in terms of Ehrlich's hypothesis—the linkage of complement to antigen by an intermediary amboceptor followed by a proteolysis in which complement acted as an enzyme—and a certain air of probability was lent to this view by the observation that the free complement in the circulating blood may be greatly diminished during anaphylactic shock. But there is no evidence that complement can play such an enzymic rôle; and the *in vitro* toxification of serum by non-specific adsorbents is clearly not explicable in such terms as these. As an alternative hypothesis it was suggested that the formation of an antigen-antibody compound, or the addition to serum of kaolin, starch or similar reagents, might remove some stabilizing constituent, perhaps antitrypsin, and so initiate a process of auto-digestion—a kind of serum autolysis. The work of Jobling and Petersen (1914) and of Bronfenbrenner (1915), among others, is in accord with this possibility; but there are almost insuperable objections to its acceptance as an explanation of the *in vitro* toxification of serum, and still more to the view that it forms the essential mechanism of anaphylactic shock *in vivo*. Thus, there has been an almost universal failure to show any relation between the increase in the toxicity of serum under the influence of a toxifying material and chemical changes indicative of proteolysis (de Kruif and German 1917, Dale 1920*b*, Doerr 1922); while the rapidity of toxification—under favourable conditions a serum may be rendered highly toxic within a few minutes—is hardly compatible with the known behaviour of proteolytic enzyme reactions. Still more is this the case in acute anaphylactic shock. As early as 1908 Wells pointed out the extreme improbability of the view that the minute amount of egg albumin that suffices to produce almost immediate shock in the sensitized guinea-pig could, in the short interval available, undergo proteolysis with the liberation of a toxic dose of degradation products. The substitution of the serum proteins for the antigen as the supposed substrate removes the quantitative difficulty, but greatly increases that introduced by the time factor. We cannot believe in the formation of an antigen-antibody compound, the adsorption by it of a stabilizing serum constituent, and a consequent digestion of the serum proteins, all occurring within a very few minutes. Moreover, substances that produce *in vitro* toxification of serum may fail to produce anaphylactic symptoms when injected intravenously into animals (Dale 1920*b*, Doerr 1922).

The exact nature of the process which underlies the *in vitro* toxification remains obscure; but it appears to be related in some way to the redistribution of the plasma constituents

that occurs during clotting (Novy, de Kruif and Novy 1917, Novy and de Kruif 1917, de Kruif 1917, de Kruif and German 1917).

Anaphylactoid Shock.—Apart altogether from the difficulties referred to above, it is very doubtful how far the acute and fatal reaction that follows the intravenous injection of a toxified serum can be identified with true anaphylactic shock. The resemblances are certainly striking, and it seems certain that common factors are operating; but there are certain differences. At necropsy the emphysema of the lungs may be rather less striking, while œdema and hæmorrhage into the pulmonary tissue are more frequent. For this reason there is general agreement that "anaphylactic" should be replaced by the ambiguous but convenient "anaphylactoid" as a descriptive term for this type of shock, and that "anaphylatoxin" should be replaced by "serotoxin" as a designation for the active agent in toxified serum.

There are, in truth, many agents other than toxified sera that may produce a fatal anaphylactoid reaction when injected intravenously into the guinea-pig. A list of such substances given by Hanzlik and Karsner (1920, 1924) includes a wide variety of organic colloids and of salts of heavy metals. The syndrome produced differs a little from one anaphylactoid agent to another; and with none does it resemble completely and in detail that of true anaphylactic shock. The spasm of the bronchioles—the most dramatic of the anaphylactic reactions in the guinea-pig—is in general a prominent feature, though it may vary in degree. There is, however, a high frequency, with these anaphylactoid reagents, of capillary thromboses and embolism in the lungs.

Among such anaphylactoid reagents—and this has been a source of error in several experiments supposed to support the humoral view—must be included all antisera that contain a high concentration of Forssman antibody (see Doerr and Pick 1913, Taniguchi 1922, Scott 1931). The toxic effect is here due, in part at least, to a direct action of the heterophile antibody on the heterogenetic antigen contained in the guinea-pig's tissues. When the serum is injected by the ordinary intravenous route the first stress of this reaction falls on the endothelium of the lung capillaries, resulting in lesions which appear to be identical with those produced by the serotoxins.

Peptone Shock.—Another anaphylactoid reagent is ordinary peptone (de Waele 1907, Biedl and Kraus 1909, Hirschfelder 1910, Doerr 1914, 1922, Dale 1920*a*, 1929, Manwaring, Clark and Chilcote 1923). The resemblance of peptone shock to true anaphylactic shock is very striking. In the guinea-pig there is the typical contraction of the bronchioles, in the dog the fall in blood pressure and the congestion of the liver. In both there may be found the loss of coagulability of the blood and the multiple small subserous hæmorrhages that are characteristic features of acute anaphylaxis in general. It is probable that peptone, like the serotoxins, acts as a direct endothelial poison (see Dale 1929). In dogs peptone shock is apparently accompanied by a release of heparin (Quick 1936) and histamine (Holmes *et al.* 1941) into the blood. After peptone shock, as after non-fatal anaphylactic shock, dogs remain in a refractory state, but peptone refractoriness depends on the degree of shock suffered, and cannot be induced by sub-shocking doses of peptone (Dragstedt 1943*a*).

Histamine Shock.—Histamine is an amine derived by decarboxylation from histidine. It was isolated from ergot by Barger and Dale (1911) and its pharmacological action has been studied in considerable detail by Dale and his colleagues, and by others (Dale 1913, 1920*a*, *b*, 1929, Dale and Laidlaw 1919, Maunter and Pick 1915, Longcope 1922, Voegtlin and Dyer 1924).

The toxic syndrome produced by histamine reproduces very faithfully several

of the characteristic features of acute anaphylaxis. In the guinea-pig the spasm of the bronchioles is constantly present in its most typical form. In the dog (Maunter and Pick 1915, Dale 1929) the muscular walls of the efferent hepatic veins are very sensitive to histamine ; in response to traces of this substance they constrict and dam back the blood in the capillary spaces of the liver. But there are also quite definite points of difference. The distension of the dog's liver with blood and lymph in anaphylaxis is far more severe and persistent than that produced by histamine. Nor does the blood become incoagulable in histamine poisoning ; but this we should expect if the incoagulability is due to the liberation of heparin.

The importance of these observations lies in the fact that histamine is a relatively simple substance of known chemical constitution, the pharmocological action of which has been studied in considerable detail. It can hardly be a mere coincidence that on intravenous injection it mimics so closely the syndrome produced by the injection of a non-toxic antigen into a sensitized animal.

Summarizing the evidence up to this point we may say that sera may be treated in various ways that render them toxic, in the sense that they produce when injected intravenously into the guinea-pig a syndrome that closely resembles, but is not identical with, that of acute anaphylactic shock. There is, however, no evidence that an " anaphylatoxin " is produced as the *direct* result of an antigen-antibody reaction occurring in the blood stream, nor has any mechanism for the production of such an anaphylatoxin been yet suggested which is compatible with all the known facts. Moreover, many reagents other than toxified sera will produce similar anaphylactoid reactions ; and one reagent of known chemical composition will give rise to some of the most characteristic features of acute shock. It seems clear that the apparent uniformity of the major symptoms of these different *in vivo* reactions is misleading, if we regard it as denoting an identity of underlying mechanism, though we can hardly be mistaken in supposing that some common actor runs through them all. It will be convenient to consider the cellular hypothesis of anaphylactic shock before discussing what this factor is likely to be.

The Cellular Hypothesis.—The essence of this hypothesis is that, for the production of anaphylactic shock, the primary antigen-antibody reaction must occur, not in the blood stream or tissue fluids, but in or on the cells of the animal. Why this cellular reaction should give rise to the striking syndrome that characterizes acute shock is a separate problem that we may consider later.

The earliest experiments made it clear that those procedures that led to a high concentration of antibodies in the circulating blood were not those that produced the anaphylactic state. Repeated injections of antigen into a normal animal lead to a high antibody content but not to hypersensitiveness. In passive sensitization the highest concentration of antibody in the blood occurs immediately after the sensitizing injection of antiserum ; but it is only after the lapse of a latent period, during which most of the antibody has disappeared from the blood stream (Fenyvessy and Freund, see Doerr 1922, Weil 1913), that hypersensitiveness is established.

Such facts as these clearly suggested that " fixed," not " circulating " antibody was concerned in the reaction. But far more decisive support for the cellular hypothesis was derived from experiments carried out with isolated tissues from actively and passively sensitized animals.

Schultz (1910) excised portions of intestine from sensitized guinea-pigs, suspended them in a bath of Ringer's solution, and studied their response to the addition of the specific antigen. He found that such isolated muscle contracted far more vigorously, when the antigen was added to the bath, than did similar preparations from normal animals when submitted to the action of similar amounts of the same foreign protein. These observations were confirmed and greatly extended by Dale (1913), using an improved technique in which one horn of the guinea-pig's uterus was substituted for the intestinal preparation, and this technique has since been used by Dale and his colleagues (Dale 1920*a*, *b*, 1929, Dale and Hartley 1916, Dale and Kellaway 1922), and by Weil (1913–17) in attempts to determine the underlying mechanism of the acute anaphylactic reaction.

The reaction of the sensitized uterine horn is extraordinarily delicate ; a typical contraction may occur after the addition of as little as 1 ml. of a 1 : 10,000,000 dilution of horse serum to a bath containing 150 ml. of Locke's solution. It is also sharply specific ; not only does the uterus removed from a guinea-pig sensitized against horse serum fail to respond to the serum of cat, rabbit, dog or man, but it is possible to sensitize separately against the euglobulin, pseudoglobulin and albumin fractions of horse serum (Dale and Hartley 1916).

All the essential features of anaphylaxis can, indeed, be demonstrated *in vitro*. Uterine horns removed from guinea-pigs sensitized either actively or passively respond specifically to the addition of the specific antigen to the bath of Ringer or Locke solution in which they are suspended. Perfusion of the uterus with warm Ringer solution before removal from the body does not abolish its sensitiveness. After a single maximal response to the addition of a relatively large amount of antigen, followed by a change to fresh Ringer's solution, the uterus no longer responds to the addition of fresh antigen : it has been specifically desensitized. If such a desensitized uterus is again washed, soaked for 2½ hours in a 10 per cent. dilution of the specific antiserum, washed in numerous changes of Ringer's solution, and again tested by the addition of antigen, it will give a typical contraction, showing that it has been passively sensitized *in vitro*. The uteri of normal guinea-pigs may be rendered hypersensitive by prolonged perfusion with diluted serum from anaphylactic or immunized guinea-pigs. This hypersensitiveness is hardly perceptible after 1 hour's perfusion, but it is easily perceptible after 5 hours' perfusion, a period strikingly similar to the latent period in passive sensitization in the living animal. Nevertheless, an almost immediate sensitization of the isolated uterus can be achieved *in vitro*, comparable in speed with that which must precede the anaphylaxis following the almost simultaneous injection of antigen and antibody into the whole animal. Kulka (1942, 1943) records sensitization of a normal uterine strip by an exposure of 1 minute to a solution of antibody, and was able to induce contractions in a normal uterine strip by adding mixtures of antigen and antibody to the surrounding fluid.

A fact of great significance was established by Dale while investigating the response of the uteri from immunized guinea-pigs. These animals had received repeated injections of horse serum increasing in amount. Some of them were injected intraperitoneally with 5 ml. of horse serum but showed no sign of shock. Nevertheless, the uterine horns, removed from other animals of the same series, reacted with a typical contraction on the addition of horse serum to the bath, thus showing that fixed antibody is present in the immune as well as in the hypersensitive animal, and lending strong support to the view that the immune guinea-pig is protected by the excess of antibody in its circulating blood.

The protective function of circulating antibody is clearly illustrated in some of Weil's experiments (see Fig. 267). A series of guinea-pigs were passively sensitized by the intraperitoneal injection of 0·1 ml. of rabbit-v-horse serum, i.e. the serum from a rabbit immunized by repeated injections of horse serum. Three days later one animal received 2 ml. of this antiserum (20 sensitizing doses) and immediately thereafter 0·01 ml. of horse serum (1–2 anaphylactic doses). This guinea-pig showed slight symptoms only. It was killed; its uterus was tested by Dale's technique, and its serum was injected into another normal

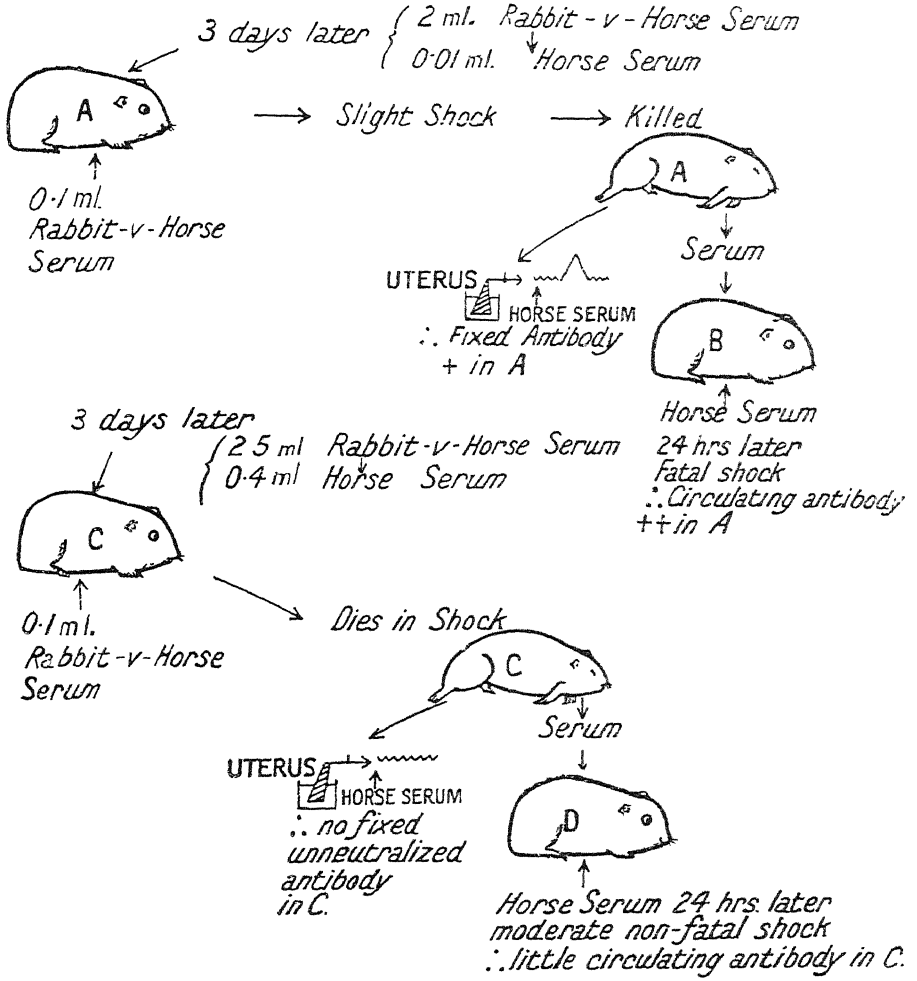


FIG. 267.

guinea-pig which was tested 24 hours later by the injection of horse serum. The uterus of the first animal gave a typical reaction, showing that it had anchored the antibody injected 3 days previously, and that this fixed antibody had not combined to any appreciable extent with the 0·01 ml. of antigen subsequently injected under cover of a protective dose of antibody. The second guinea-pig developed typical shock, showing that a considerable amount of the antibody injected into the first guinea-pig immediately prior to the injection of the antigen had remained unneutralized in the circulation. Another sensitized guinea-pig

of the same series received 2·5 ml. of the rabbit-v-horse serum (25 sensitizing doses) and immediately thereafter 0·4 ml. of horse serum (52 anaphylactic doses). It died in acute shock, showing that the amount of antibody present was not sufficient to protect the cells containing the fixed antibody from this excessive dose of antigen. The uterus, removed immediately after death, showed itself to be desensitized. A normal guinea-pig was injected with the serum of this animal and 24 hours later received an injection of horse serum. It developed moderate symptoms only, showing that the greater part of the circulating antibody in the animal that died of acute shock had been neutralized by the large dose of antigen. It should be noted, however, that serum antibody does not always protect. Morris (1936*a*) records that an excess of antibody injected into sensitized guinea-pigs led to an increase, not a decrease, of sensitivity. Nor must we in all cases attribute the anti-anaphylactic effect of intravenous sera to the presence of antibody in excess. Thus Kellaway and Cowell (1922), Morris (1936*a*), Moldovan and Maier (1941) describe an anti-anaphylactic effect in normal serum, when administered a few minutes before the shocking dose of antigen. According to Moldovan and Maier, the substance responsible for this effect is heat-stable, of low molecular weight, antagonized by pituitrin, and is produced when the reticulo-endothelial system of normal animals is stimulated by injection of colloidal material. They find that the serum of sensitized guinea-pigs is lacking in the shock-inhibitor.

Gordon (1940) records a non-specific inhibitory action of congo red in sensitized guinea-pigs, especially if administered over a period of days before the shocking dose. The mode of action of these non-specific substances is not clear. In the case of congo red, Gordon suggests that it interferes with the absorption of antigen on to sensitized cells.

Taking the evidence as a whole, the different behaviour of the hypersensitive and the "immune" guinea-pig may with some confidence be attributed to a difference in the relative concentration of fixed and circulating antibody.

If, however, we turn to other animals the evidence is more confused (see Dale 1920*b*, Scott 1931); and the observations of Manwaring and his colleagues (1923–28) on acute anaphylaxis in the dog are particularly difficult to fit into the relatively simple picture that we have drawn above. It seems, however, altogether unlikely that the mechanism of acute anaphylaxis differs fundamentally from one animal to another; and it is probable that apparent discrepancies will in time resolve themselves into the expression of secondary divergencies in a reaction of one essential type.

If we accept the view that the cellular responses that characterize acute anaphylaxis are the result of an antigen-antibody reaction occurring on or in the cells, are we to believe that this is the whole story—that it is this reaction in and by itself that affords the stimulus to cellular contraction, or produces all the localized vascular changes? If so, how can we explain the fact that those tissues that play the most dramatic part in acute shock are exactly those that are sensitive to histamine? We can hardly believe that these cells, and these alone, are concerned in the fixation of antibody.

The difficulty would in part be explained if it could be shown, as was suggested by Abel and Kubotka (1919), that histamine itself was produced by some form of protein cleavage within the cell as the result of the injury inflicted by the antigen-antibody reaction; but there is no adequate evidence that such an enzyme action occurs (see Dale 1929).

The Rôle of Histamine in Anaphylaxis.—The evidence for the essential rôle of histamine in anaphylactic shock falls under four heads. Firstly, the likeness between histamine shock and anaphylactic shock, which we have already noted; secondly, the demonstration that tissues contain histamine that can be mobilized by suitable stimuli; thirdly, the demonstration of histamine mobilization during

shock; and fourthly, the suppression of shock by measures that destroy or inhibit histamine or inhibit the reaction.

Lewis (1927), in his classical studies on the reaction of the skin to a localized stimulus or injury, was able to show that the local reddening, due to the dilatation of the minute blood vessels, and the succeeding local wheal, were independent of the local neuro-vascular mechanism, whereas the surrounding flare, due to the dilatation of the neighbouring arterioles, was dependent on a local axon reflex. He concluded that the reddening and the wheal formation were the result of a chemical stimulus provided by some substance liberated from the injured cells; and he found that histamine, alone among the many known substances that he tested, reproduced in complete detail the effects of this cell constituent, which he refers to as the *H-substance*. Dale and his colleagues (see Dale 1929, Dale, Dudley and Thorpe 1927, Harris 1927, Thorpe 1928) showed that histamine is a normal constituent of many different tissues; and the evidence available strongly supports the view that it exists as such during life, and is not formed from some mother-substance after cell-death. Whether it is present in the free uncombined state, in which it is readily diffusible, is more doubtful. There are reasons for believing that it may, in certain circumstances at least, be present, and be liberated from the cell, in loose combination with cell-constituents of high molecular weight and low diffusibility.

The relative histamine content of different tissues is interesting and suggestive. Using a physiological method of assay (see Thorpe 1928, Harris 1927) the lungs have been found to contain between 35 and 75 mgm. of available histamine per kilo, the liver between 2·5 and 6·6 mgm., the skin as a whole about 6 mgm. (24 mgm. in the epidermis and 4 mgm. in the dermis). Other tissues contain much less. The voluntary muscles contain about 1 mgm. per kilo, the testis about 1·8 mgm., the submaxillary gland about 0·5 mgm., and the thyroid about the same.

Histamine is destroyed rapidly in normal blood, a fact which probably accounts for early failures to detect this substance in anaphylactic animals (see Dragstedt and Mead 1935). Dragstedt and Mead (1936*a*) described a histamine-like substance in the blood of dogs after anaphylactic shock. By measuring the normal rate of destruction of histamine in the blood, they calculated the amount present in the animals at the onset of shock, and found it enough to account for the vascular reactions of the shocked animal. The substance was specifically destroyed by the enzyme histaminase (Dragstedt and Mead 1936*b*; see also Code 1939).

Watanabe (1931) obtained some evidence that the histamine content of the lungs of sensitized guinea-pigs was abnormally high, and much less in the lungs of animals after shock. Bartosch, Feldberg and Nagel (1932) noted the appearance of a histamine-like substance in the perfusate of sensitized guinea-pig lungs when antigen was added to the perfusion fluid. This observation was confirmed by a number of workers, and the identity of the substance with histamine more firmly established (see Schild 1937). Increased quantities of the histamine-like substance were also found in the blood of the whole guinea-pig after shock (Simon and Staub 1937, Code 1939). In the rabbit, on the other hand, though anaphylactic shock and histamine shock are similar, a drop in the blood histamine after shock was observed by Rose and Weil (1939). Rabbit's blood, however, is rich in histamine, a fact which led Katz (1940) and Dragstedt, Ramirez de Arellano and Lawton (1940) to test the *in vitro* effect of antigen on the blood of a sensitized rabbit. Both found that histamine was liberated from the leucocytes into the plasma. (See also Rose and Browne 1940.) Dragstedt (1941) suggests that in the intact sensitized rabbit the injection of antigen leads to the accumulation of the histamine-rich leucocytes in the lung (where it is perhaps liberated), and a consequent fall in blood histamine.

There is thus good evidence that cellular injury, whether anaphylactic or, as in peptone shock, anaphylactoid, in type is liable to liberate histamine into the tissues. We do not know in anaphylaxis whether the cells that are injured and

liberate histamine are themselves histamine-sensitive, whether minimal injury may so disturb the internal economy of a cell that it becomes reactive to its own contained histamine, or whether we should locate the injury and liberation of histamine in one type of cell, the response to the liberated histamine in another; these are problems which are not soluble at present. In some cases there are suggestive hints. Isolated plain muscle from the sensitized dog gives no significant response to the specific antigen. Yet the plain muscle of the dog is sensitive to histamine. It would seem that in the dog, as opposed to the guinea-pig, the sensitization to the antigen is limited mainly to the liver.

We may now consider briefly the effect of anti-histamine substances on anaphylactic shock. Histamine is readily destroyed by the enzyme histaminase (Best and McHenry 1930). However, though Karady and Browne (1939) claimed to have protected sensitized guinea-pigs from shock by intravenous injections of the enzyme 15 minutes before the shocking dose, their findings were not subsequently confirmed (Knoll 1940, Rose and Browne 1941, Younger, Freedman and Nungester 1941, Alexander and Bolton 1940). Staub (1939) described an *in vitro* anti-histamine action in a number of synthetic amine bases. One of these, N-phenyl-N-ethyl-N′-diethylethylenediamine, was reported by Wilcox and Seegal (1942) to have inhibited anaphylactic shock in sensitized guinea-pigs.

The administration of sublethal doses of histamine induces a state of increased tolerance to the drug. Farmer (1939) and Karady (1941) claim that histamine desensitization is accompanied by a moderate degree of desensitization to specific anaphylactic shock. Fell and others (1943, Rodney and Fell 1943) immunized rabbits with horse globulin coupled with the pharmacologically inactive *p*-aminobenzoyl histamine, and induced the formation of antibodies whose specificity was partly determined by the histamine. The immunized animals were then sensitized with ovalbumin. As compared with non-immune sensitized controls, there was definite evidence of a reduced liberation of histamine into the blood during shock, and of a reduced skin sensitiveness either to shocking doses of antigen, or to histamine. Immunized guinea-pigs, subsequently sensitized to ovalbumin, were protected to a marked degree against fatal shock. It was considered unlikely that the protection was due to desensitization by histamine liberated from the globulin antigen. As the authors point out, if the protection is due to specific antibodies against histamine, it remains to determine how such antibody could act in anaphylaxis, and yet have no observable effect on the normal histamine metabolism in the body.

The Rôle of Acetyl-choline in Anaphylaxis.—Went and Lissak (1936, 1938) and Martin and Went (1939) have reported that during the perfusion of the heart of a sensitized guinea-pig with antigen solution, a substance resembling choline is liberated from the muscle, and that the heart muscle of sensitized guinea-pigs is more sensitive than normal hearts to the pharmacological action of choline. Went suggested that choline and acetylcholine, as well as histamine, may be liberated from different organs during shock. Wenner and Buhrmester (1937) found acetylcholine in the blood stream of rabbits after shock, but Ratnoff (1939) could not confirm their findings. Kourilsky and his colleagues (1938) detected no acetylcholine in the hæmorrhagic lesions of shocked animals. They noted that acetylcholine produced no hæmorrhages in the tissues on local injection, whereas histamine did so. Nakamura and his colleagues point out that acetylcholine shock and anaphylactic shock have many features in common, and few in common with histamine shock, both in symptomatology and modification by physostigmine, eserine and other drugs. They attributed the main symptoms of anaphylactic shock to acetylcholine liberated as the result of antigen-antibody union in the region of the nerve-endings of the sympathetic system (Nakamura and Takahashi 1938, Nakamura 1941, Chigira 1941). Farber, Pope and Landsteiner (1944) were able regularly to demonstrate histamine in shocked guinea-pig lung; only in shocked heart tissue was acetylcholine in excess of the normal value, and even there the increase was demonstrable only in 10 per cent. of the tests (see also Bender 1944). The evidence

as a whole points to a participation of choline or acetylcholine in shock, but only as a minor factor.

We may then, in general terms, describe the acute anaphylactic reaction as consisting of two different but related effects—cell injury determined by an antigen-antibody reaction occurring in or on those cells by which antibody has been fixed, and a series of secondary effects due to the liberation of various substances among which histamine, or a histamine-like substance, appears to predominate. The varieties of secondary effects are determined by the response of the cells sensitive to histamine and the other substances liberated. Among the cells capable of liberating histamine under the influence of an antigen-antibody reaction, the possible participation of the circulating leucocytes should be noted.

As regards the anaphylactoid reactions, they differ from acute anaphylactic shock in that the cell injury is not determined by an antigen-antibody reaction; they resemble it in that the secondary histamine effect is essentially the same. It should be noted that most of the anaphylactoid reagents, such as toxified sera, do not stimulate contraction of the isolated guinea-pig uterus. This histamine-liberating injury presumably occurs elsewhere when these agents are injected *in vivo*. Peptone, on the other hand, has a direct stimulating effect on plain muscle, which suggests that it initiates a mobilization of histamine.

The complete picture of an anaphylactic or anaphylactoid reaction will thus consist of a description of cellular injury, differing from case to case according to the reagent employed and the susceptibilities of the experimental animal, and a histamine effect, differing from one animal species to another according to the distribution of the histamine-sensitive cells, but common to all reactions in one animal species, by whatever reagents they are caused.

Local Anaphylactic Reactions

Within a year of the original observations of Portier and Richet, and before the phenomenon of acute anaphylactic shock had been submitted to any detailed study, Arthus (1903) described a curious reaction in the rabbit, which was soon recognized as belonging to the same order of exaggerated response to a foreign antigen. Inoculating rabbits subcutaneously with repeated doses of horse serum, he noted that the initial injections were without any detectable effect, but that, as one injection succeeded another with suitable intervals between them, the local reactions became more intense and more persistent. After a few injections the site of inoculation became swollen and œdematous. These local swellings were at first transient, but with each successive injection they lasted longer, with more definite induration, until finally the response took the form of a firm indurated swelling which progressed to a localized necrosis. This altered response was not confined to the tissues in the immediate neighbourhood of the earlier injections. The rabbit, as a whole, had become so sensitized that it reacted in this characteristic and abnormal way to the injection of the specific antigen at any site (see also Seegal, Seegal and Jost 1932, Davidoff, Seegal and Seegal 1932). It may be noted that this reaction, in its typical form, appears to be peculiar to the rabbit. Nicolle (1907) failed to reproduce it in the guinea-pig, and Opie (1924) failed to elicit it in either the guinea-pig or the dog. Dragstedt (1941) suggests that the peculiar susceptibility of the rabbit may be due to the high histamine content of its leucocytes, which, accumulating in the early tissue response to the local injection of antigen, form a rich source of local histamine by the time sensitization has occurred.

HYPERSENSITIVENESS IN MAN

Clinical observations have long been on record containing descriptions of reactions in human subjects that present obvious analogies to the anaphylactic reaction in experimental animals.

Serum Sickness.—It has been known for many years that a proportion of those persons who receive injections of antitoxic or antibacterial serum for prophylactic or therapeutic purposes develop a curious and characteristic condition which has been given the name of " serum sickness." An excellent description of the disease and a detailed discussion of its significance are contained in the monograph of von Pirquet and Schick (1905). The great extension of serum therapy in modern times has provided the data for large-scale surveys of the incidence and kinds of serum sickness (see Park 1921, 1928, Beer 1938, Lyall and Murdick 1938, Davis 1938, Rutstein *et al.* 1941, Kojis 1942). For our immediate purpose we need refer only to the following points.

The reaction must be distinguished from the disturbances of the thermo-regulatory mechanism, and the cardio-vascular reactions which are commoner sequels to the injection of serum. It occurs after primary as well as after subsequent injections. It is in no way related to the antibody content of the injected serum, but is a response to the foreign protein as such, which is usually horse serum. The reaction may occur within 2 hours of the injection of the serum, and up to 24 days after, though an incubation period of 8 to 12 days is commonest. Among the " immediate " reactors, there is often evidence of previous treatment with horse serum. Younger people are more prone than older, though the proneness may reflect the fact that younger persons are more liable to have had previous antiserum treatment, as compared with persons whose youth was passed in an age of less extensive serum therapy. The illness itself is characterized by rashes, especially of an urticarial type and often commencing at the site of the inoculation, by fever, joint pains, slight œdema without albuminuria (or with a mere trace of albumin in the urine), and by a varying degree of glandular swelling usually confined to the regional glands that receive the lymphatics from the site of inoculation. The illness is variable in duration, protean in its symptoms, and seldom of a serious nature, though it may be associated with severe discomfort. It is probably never fatal. The nervous system is affected, though rarely. The commonest lesions occur in the 5th and 6th cervical nerves. Neuritis is followed by flaccid paralyses of the muscles, with a slow and, in the greater proportion of cases, a complete recovery. Both motor and sensory nerves are affected. The neuritis may affect one or many nerves, and in some cases the lesions are more central and may even be cerebral. The neuritis is ascribed to an urticarial œdema of the nervous tissue. It should be noted that these paralyses may follow the injection of other antigenic substances—vaccines and preparations of toxoid (see Kennedy 1929, Allen 1931, Young 1932, Doyle 1933, Ayer 1935, Wilkinson 1937, Bennett 1939, Hughes 1944).

In a tiny minority of cases an entirely different reaction follows the injection of serum, the patient dying within a few minutes with symptoms resembling those of acute anaphylactic shock. Park (1908) records two such cases among 50,000 persons who received serum injections in New York (see also Park 1921, 1928, Vance and Strassman 1942).

Acute anaphylactic shock can also follow the injection of antigens other than serum proteins. There are, for example, a number of cases on record of shock after a second dose of a bacterial toxoid; the peptone used in the preparation of the toxoid appears to have been the responsible agent (see Parish and Oakley 1940).

We may note that processes which destroy the integrity of the antibody globulin molecule in antisera from the horse also reduce their capacity to cause serum reactions. Thus, when proteolytically refined antisera (Chapter 7) are employed,

the incidence of serum sickness and of sensitization to serum sickness, are reported to be greatly reduced (Pöhacker-Fritsch and Siegl 1941, Kojis 1942; see also Coghill *et al.* 1940, Jones and Roberts 1941, Top and Watson 1941).

The Idiosyncrasies.—Another group of reactions, which clearly come into the general category of hypersensitiveness, are the so-called idiosyncrasies, in which certain persons develop a characteristic symptom-complex as the result of natural contact with some particular substance, or range of substances. The substances that may evoke this response are of the most varied nature: in hay fever, pollens of various species; in asthma, pollens, horse dander or the cutaneous débris from other animals; in the food idiosyncrasies, a wide range of different materials; in the drug idiosyncrasies, a whole series of chemical compounds which seem to have no characteristic or common structure.

One of the most striking features of the idiosyncrasies as a group is that—as in the anaphylactic reaction—the symptom-complex bears no relation to the particular type of substance by which it is evoked. In spite of the varied nature of the exciting materials concerned, the syndrome induced shows an essential uniformity; though there are minor differences which seem to depend in the main on the particular route by which the reacting substance gains access to the tissues. It is particularly striking that, in the case of drugs, the response exhibited by the hypersensitive subject—using hypersensitive in this particular sense—bears no relation to the pharmacological action of the drug in question.

The reactions induced may be either local or general. Among the former are the local reaction to the intracutaneous injection of horse serum or to the application to the skin of such a drug as iodoform, the coryza of hay fever and perhaps the associated asthma, and the acute vomiting and gastro-intestinal disturbance that follow the ingestion of a particular food-stuff. Among the latter are the generalized urticarial eruptions or œdema that may follow the administration by any route of a substance to which a person is hypersensitive, the occasional pyrexia, and the asthmatic symptoms that sometimes follow the entry of the exciting substance by a route other than the respiratory tract.

There are obvious points of similarity between this group of reactions on the one hand and anaphylactic hypersensitiveness on the other, but there are also obvious divergencies.

Among the points of similarity we may include the following (see Coca 1920*a*):—

In both cases the reaction is specific. Although the naturally hypersensitive person may react to several different substances the range is usually limited, and may be confined to a single chemical substance. In both cases the type of response is essentially the same for a single animal species irrespective of the nature of the exciting substance.

Among the features that have been held to differentiate between the two conditions are the following. The exciting agent in anaphylaxis is always an antigenic substance; the exciting agent in human hypersensitiveness is often non-antigenic. Anaphylactic hypersensitiveness is induced by the injection of a sensitizing dose of antigen; hypersensitiveness in man is often exhibited upon first contact with the exciting agent. The anaphylactic state, apart from congenital passive anaphylaxis, is not a heritable condition; natural human hypersensitiveness shows a definite tendency to run in families, though the hypersensitiveness is often exhibited towards different substances in parents and offspring. Specific desensitization is far more easily demonstrated in anaphylaxis than in hypersensitiveness.

Taking these points of difference *seriatim* we may attempt to assess their significance in the light of the evidence now available. Coca and his colleagues, who would regard the differences between the two conditions as sufficient to justify a formal separation into different categories, employ the term *atopy* to denote the natural hypersensitive state, and the term *atopen* to denote the exciting agent (see Coca and Cooke 1923). We may define our first problem as the determination of the differences between atopens and antigens.

Atopens and Antigens.—It is certain that many substances are at the same time antigenic and atopic. Horse serum forms an excellent example, though we should note that Coca and Cooke separate serum sickness from the true atopies. If, however, we confine ourselves to the more typical atopens we still find that a single substance commonly displays both types of activity.

Alexander (1924) found that guinea-pigs could be rendered anaphylactic to rye pollen, though he could not induce the formation of precipitating or complement-fixing antibodies in detectable amount. Parker (1924) reports the production of precipitins for ragweed pollen in rabbits and guinea-pigs, and Ramsden (1926) obtained similar results. Walzer and Grove (1925) showed that the pollens of timothy-grass and ragweed have definite antigenic properties, since both induce the anaphylactic state in guinea-pigs, demonstrable either by intravenous injection or by Dale's technique. They note, however, that sensitization with pollens is far more difficult and uncertain than with such an antigen as horse serum, and they failed to demonstrate precipitins in the serum of the sensitized animals. Caulfeild and his colleagues (1926) prepared various fractions from ragweed, timothy-grass and birch pollens, and obtained with them active and, in some cases, passive sensitization. Ramsden (1926) demonstrated both active and passive sensitization of guinea-pigs to pollen. Longcope and others (1925, 1927) have demonstrated the active sensitization of guinea-pigs to extracts of horse dander, and have succeeded in specifically desensitizing such animals by the usual procedures. Spies and his colleagues have fractionated both cotton seed and kapok seed, and find a number of components capable of sensitizing and shocking experimental animals. The active components are mainly protein in nature, though associated in some instances with carbohydrate. It is noteworthy that the degree of the antigenic relationships between the two seeds in the main reflected the observed degree of cross-reactivity of the two as atopens in sensitized persons (Spies *et al.* 1939, 1940, Coulson and Spies 1943, Coulson *et al.* 1943, 1944, see also Newell 1942). There seems little doubt that many of the reagents that have been classed as typical atopens have a definite antigenic action.

There is, of course, no doubt at all that many of the substances that function as atopens—for example many drugs—are non-protein in nature and are certainly not antigenic in the full sense. Moreover, it has been found (Grove and Coca 1925) that the removal of all proteins and other detectable nitrogenous substances from extracts of pollen, or of house dust, does not cause any appreciable loss of atopic activity, as tested by intracutaneous inoculation in susceptible persons. On the other hand, the tryptic digestion of the proteins of horse dander, or of the green pea, caused a loss of atopic activity corresponding with the loss of protein.

The fact that non-protein substances can excite a specific response in the hypersensitive person cannot, however, be taken as a proof that antigens and atopens differ in any essential respect. We have seen that many haptens, when injected intravenously into actively or passively sensitized guinea-pigs, will induce acute anaphylactic shock, so that it would be in no way surprising if a fundamentally

similar reaction were produced in naturally sensitive persons by contact with antigenic components of a similar kind.

There remain instances of drug idiosyncrasies that are not so readily explicable along these lines, since the chemical structure of the exciting agent is not of the relatively complex kind which characterizes those haptens that are capable of inducing acute shock in a sensitized animal.

Recent observations have, however, indicated a possible mechanism by which such simple compounds might come to function as complete or partial antigens. It has been found that animals may be sensitized by the injection of atoxyl and serum, or even of arsenical compounds alone (see Klopstock and Selter 1929, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931); and it seems probable (Landsteiner and Levine 1930) that in such cases a preliminary union occurs between the simple arsenical compounds and some serum or tissue protein, with the formation of an effective antigenic complex. In later studies Landsteiner and Jacobs (1936) sensitized guinea-pigs with neoarsphenamine, and induced shock by the intravenous injection of guinea-pig serum mixed with arsphenamine, or arsphenamine alone. Jacobs (1932) succeeded in sensitizing guinea-pigs to iodinated sera by injecting them with guinea-pig serum mixed with Lugol's iodine solution, and suggested that a similar mechanism might be involved in the development of iodine sensitiveness in man. Horsfall (1934) injected formolized rabbit serum into rabbits, and found that the animals developed skin sensitivity to uncombined formaldehyde. Landsteiner was able to induce both skin sensitivity and anaphylactic sensitization by the intracutaneous injection of aromatic substances like picryl chloride and dinitrochlorobenzene (Landsteiner and Jacob 1936, Landsteiner and Chase 1937) and non-aromatic substances like diazomethane and mustard oil (Landsteiner and di Somma 1938). These substances are all highly reactive and capable of forming conjugates with tissue proteins, and are also known to induce dermatitis or other forms of hypersensitivity in man (see, for example, Wedroff 1932; see also Landsteiner and Chase 1939, 1940*b*). From a study of the sensitizing action of tetryl (trinitrophenylmethylnitramine) in the skin of guinea-pigs, Gell (1944) concluded that this substance also acted by forming a picryl protein in certain tissues; though there was evidence that not all compounds of tetryl and guinea-pig protein would stimulate sensitivity, and that sensitivity was not necessarily associated with the presence of circulating antibodies to picryl protein.

Para-phenylenediamine also induces sensitivity when applied to the skin of guinea-pigs, and Nitti, Bovet and Depierre (1937) record an interesting series of observations on the specificity of the sensitization with regard to the structure of the substance. Thus, the corresponding *ortho*- and *meta*-compounds would not sensitize, and substitution of SO_2NH_2, $—CH_3$ or —OH groups for one of the $—NH_2$ groups entirely destroyed the allergic capacity of the *para*-compound. Various other substitutions decreased or enhanced both activity and specificity, in a manner which recalls the high degree of dependence of antigenic specificity on chemical structure (see Chapter 7).

Active and Passive Sensitization in Man.—The second criterion that has been accorded differential significance is the mode of sensitization. There is no question that human hypersensitiveness occurs naturally and apart from any known previous contact with the particular antigen to which the sensitive subject reacts, just as the "natural" antibodies may be found in persons who, so far as we can tell, have never been infected with or immunized against the particular bacterium on which their sera react.

There is, however, good evidence that this condition frequently arises as the result of active sensitization.

Hooker (1924) tested a large number of persons by the intracutaneous injection of horse serum before and after they had been immunized by the injection of toxin-anti-

toxin mixtures. Of 367 persons tested before immunization 308 failed to react to horse serum. Of these, 100 were found to be Schick-positive and were immunized by toxin-antitoxin injections. On retesting these 100 persons at a later date, 66 gave positive reactions to horse serum. Park (1924) carried out a similar series of observations, in which he tested the sensitiveness to horse serum of immunized and non-immunized groups of children. He found that 84 of 116 children who had received toxin-antitoxin injections reacted positively to horse serum, while of 90 children who had not been immunized 45 were sensitive, giving a difference of 22·5 per cent. between the two groups. Other observers (Cowie 1914, Longcope and Rackeman 1918, Gordon and Creswell 1929, Mackenzie 1924) have obtained results that point in the same direction. It would appear that the injection of horse serum significantly increases the percentage of sensitive persons in a sample of the normal population. As we have already noted (p. 1119), the intradermal injection of pneumococcal polysaccharides may induce a specific skin-sensitivity, so that this effect is not limited to protein substances.

Again (see von Pirquet and Schick 1905), while the serum sickness that develops in a naturally hypersensitive person, apart from previous sensitization, is almost always of the delayed type described above, a proportion of those cases that occur in persons who have received previous injections of horse serum show a characteristic acceleration of the response. In some cases the localized œdema, the generalized urticaria, the febrile reaction and all the symptoms of severe serum sickness may develop within 24 hours.

The most striking evidence in favour of the view that experimental anaphylaxis and natural hypersensitiveness depend on essentially similar mechanisms has, perhaps, been provided by the demonstration that hypersensitiveness to a particular atopen or antigen can be passively transferred from one person to another, since this clearly places human hypersensitiveness with anaphylaxis in the category of the antigen-antibody reactions.

Ramirez de Arellano (1920) reported a case of sensitiveness to horse dander. The man was employed as donor in the transfusion of an anæmic patient who received 600 ml. of his blood. The recipient, who gave no history of such an idiosyncrasy, thereafter developed marked hypersensitiveness to horse dander. The evidential value of this case is somewhat lessened by the fact that the same donor was used in another transfusion without rendering the recipient hypersensitive. Again, serum sickness is associated with the appearance of precipitins in the blood, either during the sickness, or after the height of the disease (Longcope and Rackemann 1918, Jones and Fleisher 1932, DeGara and Bullowa 1942), and transfer of the serum will induce a passive anaphylactic state in the guinea-pig. Recently Karelitz and Stempien (1942) produced " passive serum sickness " by injecting into persons, already treated with therapeutic horse antisera, the serum of other persons who were convalescent from serum sickness.

An observation that has provided a basis for the experimental study of passive sensitization in man was recorded by Prausnitz and Küstner in 1921. Küstner himself was markedly sensitive to the cooked flesh of certain fish. Using an extract of cooked fish as antigen these observers failed to demonstrate the presence of precipitins in Küstner's serum, nor could they produce passive sensitization of guinea-pigs. If, however, a little of Küstner's serum was injected into the skin of a normal person and the antigen was injected into the same site 24 hours later, a marked local reaction was produced. This reaction did not occur if the antigen and the serum were injected simultaneously, a finding strikingly analogous to the latent period in the passive anaphylactic sensitization of the guinea-pig. The

Prausnitz-Küstner reaction has been used extensively for the study of passive sensitization in man. Wright and Hopkins (1941) describe a more sensitive reaction, depending on "reversed" passive sensitization, in which the skin site is injected first with antigen, and, 24 hours later, with the serum.

The detailed study of this passive atopic sensitization has been taken up by Coca and his colleagues and by other investigators. Coca and Grove (1925) gave the name *atopic reagins* to the antibodies present in the serum of naturally hypersensitive persons, through the agency of which the specific hypersensitiveness can be passively transferred to the skin of normal non-sensitive persons. They record that these reagins have been demonstrated in the serum of all those subjects of hay fever or of asthma in whom the reaction to the intradermal injection of the specific atopen is positive. They find, however, that not all normal skins are receptive, about 11 per cent. failing to react at all, and some 5 per cent. giving slight or doubtful reactions. The remaining 84 per cent. are fully receptive, and the local passive sensitization lasts for at least 4 weeks once it has been established. They find that the reagin may be neutralized by the specific atopen in the test tube, but that it gives no precipitin reaction *in vitro*, nor will it induce passive anaphylaxis in the guinea-pig. In a control series of experiments they noted that the normal human skin could not be passively sensitized to egg white or to ragweed-pollen with rabbit antisera, although these contained specific precipitins. Exactly similar phenomena have, however, been noted in the case of passive anaphylaxis—neither horse antisera nor chicken antisera are effective in the passive anaphylactic sensitization of the guinea-pig, though antisera prepared in the rabbit are highly efficient from this point of view.

Clarke and Gallagher (1926) give a very clear-cut demonstration of the latent period in passive atopic sensitization and of the *in vivo* neutralization of reagin by atopen without the production of any tissue reaction. They injected 0·05 ml. of atopen into one area of skin, and 5 minutes later they injected 0·05 ml. of reagin-containing serum into the same site and 0·05 ml. of the same serum into a second site some distance from the first. No reaction occurred after these injections, showing that the union in the tissues of atopen and reagin under conditions that give no time for the preliminary fixation of the reagin does not produce the typical response. Next day each site was reinjected with the same quantity of atopen. At the second site, where no atopen had previously been injected, a typical reaction occurred. At the first site no obvious response was elicited, showing that the reagin had been neutralized by the atopen that was present when the sensitizing injection was given.

Levine and Coca (1927) found that there was a proportional relationship between the degree of sensitivity of the skin and the reagin content of the serum in 33 cases of hay fever.

Reagins and Antibodies.—The observations quoted above do not suggest that any clear separation can be made between these two types of antibody. It is true that reagins may be demonstrated in sera that give no precipitation with the specific antigen; but, as we have seen, passive anaphylaxis may be induced with sera in which no precipitins can be detected. Again, sera that contain antibodies may not induce passive sensitization. Karelitz and Glorig (1943) record an instance of a serum from a patient convalescent from serum sickness, which was rich in anti-horse precipitin but which, on intravenous injection into a normal subject, failed to react with an antigen injected locally in the skin. Therapeutic horse serum, however, is not a single defined antigen, and we cannot exclude the possibility of two different antibodies, only one of which is capable of passive sensitization. It is by no means clear, therefore, that reagins and precipitins are different antibodies; the apparent qualitative differences may in reality be quantitative—as they appear to be in anaphylaxis—depending on a difference in delicacy between

the *in vivo* and *in vitro* tests. De Besche (1918), for instance, has reported the presence of precipitins in the serum of a considerable proportion of a small group of asthmatics who were sensitive to the proteins of the horse, cat, dog or cow. He notes that patients who showed the presence of precipitins at one time might fail to do so at another. The antibodies appeared to fluctuate above and below the level at which they were demonstrable *in vitro*. Moreover, the precipitin reaction is relatively insensitive for the detection of antibody (see Chapter 7). Cannon and Marshall (1941) devised a more sensitive test for antibody, using the agglutination of collodion particles coated with antigen. By this means, they found a marked parallelism between serum antibody and sensitivity to the Arthus phenomenon in rabbits, both before and after desensitization.

Otto and Adelsberger (1931) have recorded a careful series of observations on the relation between the atopic reagins and the anaphylactic antibodies. They note that the typical reagin, as exemplified in the sera of persons sensitive to horse dander, is extremely effective in the transference of specific sensitivity to a localized area of the normal human skin. It is less effective in sensitizing the skin of the guinea-pig, and quite ineffective in sensitizing that animal to anaphylactic shock. The sera of persons suffering from serum sickness, or of those who have been injected with horse serum without suffering from the clinical disease, are effective—though somewhat less effective—in the transference of sensitivity to the normal human skin. They are far more effective in sensitizing the guinea-pig's skin ; and are not infrequently effective in inducing generalized anaphylactic sensitization. Anti-horse sera, prepared in rabbits or guinea-pigs, are relatively—but not entirely—ineffective in the transference of sensitivity to the human skin, but are uniformly effective in inducing passive anaphylaxis in the guinea-pig. Again, Caulfeild, Brown and Waters (1937) were able to induce the same specific sensitization to extract of ragweed pollen in the skin of a normal person by serum from a hypersensitive person, and by serum from a guinea-pig immunized with ragweed extract.

It is possible that the passive transfer of reagins is most easily accomplished when the donor and recipient of the serum are biologically related. Straus (1937), for instance, found that *rhesus* monkeys were readily sensitized by the injection of human sera containing reagins.

The evidence as a whole suggests minor differences in the antibodies with which these various workers have been concerned—differences perhaps connected with the proteins in antisera obtained from different animal species rather than with the active grouping of the antibody ; but there seems no adequate reason for regarding reagins and anaphylactic antibodies as belonging to essentially different types. *Atopens* and *reagins* might well give place to *antigens* and *antibodies* in immunological nomenclature, except where some convenient term is required to denote the way in which an antigen or antibody is functioning at a particular time.

The substances that induce natural hypersensitivity are complex. The various manifestations of hypersensitivity to a substance may therefore be the result of interactions between more than one kind of antibody and perhaps more than one kind of antigen. We have already seen that antibodies vary in their powers of inducing passive sensitization, and that one antigenic preparation may have conspicuously different powers of sensitizing on the one hand and inducing an allergic reaction on the other. The different reactivities of sera from hypersensitive persons may alter independently of one another.

For instance, Schmidt and Lippard (1937), who measured the capacity of " hypersensitive " sera to neutralize the corresponding antigen so that it would no longer elicit a

Prausnitz-Küstner reaction, and the capacity to sensitize the skin of normal persons, found that the latter was much more readily destroyed than the former, by heating to 56° C. In this case we cannot tell whether a single antibody was altered in respect of two activities or whether two antibodies were concerned. There is, however, evidence that more than one type of antibody is concerned in certain manifestations of hypersensitivity. Cooke (1941) showed that the desensitization of persons sensitive to certain pollens was accompanied by the appearance in the serum of an antibody capable of neutralizing the pollen antigens. This " blocking " antibody appeared to be distinct from the reagin upon which the passive transfer of hypersensitivity depended. For example, Sherman, Hampton and Cooke (1940) found that blocking antibodies in pregnant mothers were transmitted to the child *via* the placenta, but that reagins were not. However, neither Cooke nor Scully and Rackmann (1941) could find any correlation between the titre of blocking antibody and the state of desensitization of the patient. Loveless (1940, 1941, 1942, 1943), on the other hand, believes that the reagin is responsible for sensitivity, and the blocking antibody for the desensitized state. She finds this to be produced specifically by injection of pollen ; it is non-sensitizing, and readily diffusible, and differs from reagin in being relatively thermostable, withstanding exposure to 56° C. for 24 hours.

The Genetic Factor in Anaphylaxis and Hypersensitiveness.—The third character that has been held to differentiate human hypersensitiveness from anaphylaxis is concerned with the part played by inheritance in the two conditions. There is evidence (Cooke and Veer 1916, Coca 1920*a*, Spain and Cooke 1924, Clarke, Donnally and Coca 1928) that human hypersensitiveness tends to run in families. There is at present no evidence that the anaphylactic state, as induced in the sensitized guinea-pig, occurs naturally or is determined by genetic factors. Does this indicate any essential difference in the nature of the two phenomena ?

Even if we accepted the premises as warranting such a conclusion we should require much more evidence than we have before we accepted the second premise as valid. Anaphylaxis in its usual experimental form is, of course, never inherited ; since one of the necessary conditions for its study is that the injection of the antigen into the unsensitized animal shall not produce shock, *i.e.*, the experimental animal selected must not be naturally sensitive to the antigen under test. Whether there are any cases in which the injection of an antigen into an animal that has not been artificially sensitized produces typical anaphylactic shock, demonstrably due to an antigen-antibody reaction, we do not know, because no systematic search has ever been made. In the absence of such knowledge we cannot tell what part inheritance plays in the natural anaphylactic state, if that state occurs. As Doerr (1922) points out, we have for obvious reasons studied natural hypersensitiveness in man and anaphylaxis in experimental animals. It is at least possible that the differences noted are referable in large part to the different fields that have been selected for study.

With regard to the inheritance of human hypersensitiveness we have already noted (see Cooke and Veer 1916, Spain and Cooke 1924) that the genetic factor appears to be concerned with a tendency to develop atopic hypersensitiveness in general, rather than a sensitiveness to any one atopen in particular. It appears to be unusual to find that parents and offspring, or several members of the same family, are all hypersensitive to the same substance ; one may respond to ragweed pollen, another to horse dander, another to cat hairs, and so on. It has also been noted (Clarke, Donnally and Coca 1928) that the type of reaction to atopic substances in general appears to be in some degree inherited ; the tendency to develop asthmatic symptoms, for instance, being particularly frequent in certain families.

Landsteiner and Chase (1940*a*) report that guinea-pigs can by breeding be separated

into strains of high and low susceptibility to sensitization by dinitrochlorobenzene, or to the atopens of poison ivy, thus affording one indication of a similarity between man and experimental animals in a heritable tendency to develop hypersensitivity.

There seem insufficient grounds for postulating an essential difference between anaphylaxis and hypersensitiveness on the premises of our present knowledge of the influence of genetic factors in the two conditions.

Desensitization in Hypersensitiveness and Idiosyncrasy.

Fourthly, some observers, and particularly Coca (1920*b*), would lay great stress on the difficulty of desensitization in the sensitive human subject as compared with its relative ease and certainty in the anaphylactic animal. While admitting that a degree of lessened sensitivity to natural contact with the specific atopen may be attained in a hypersensitive person by suitably graded injections, Coca points out that the further administration of the atopen by subcutaneous or intracutaneous inoculation will always demonstrate the persistence of the hypersensitive state.

It seems doubtful, however, whether this criterion has any greater differential value as between the atopic and anaphylactic states than those we have discussed above. It is true that desensitization may readily be demonstrated in the anaphylactic guinea-pig ; but even here it depends on a strict observance of the necessary quantitative and time relationships, as may be seen in many of Weil's experiments. In other species the problem is far less simple ; it is for instance notoriously difficult to desensitize the rabbit.

In the hypersensitive human subject, as in the experimentally sensitized animal, the available evidence indicates that desensitization induces a temporary change in the immunological balance of the tissues, with a subsequent reversion to the hypersensitive state (see Vallery-Radot *et al.* 1936, Adelsberger 1936, Gernez 1936).

We cannot of course apply the results obtained in our animal experiments, without modification, to our clinical problems. The relief of a case of hay fever or the desensitization of a serum-sensitive patient as a preliminary to the administration of antitoxin, demands the application of a technique that can be elaborated only on a basis of empirical trial, and which may or may not be successful. This does not, however, justify the conclusion that there is any essential qualitative difference in the underlying mechanism.

ALLERGIC REACTIONS IN BACTERIAL INFECTIONS

The term *allergy* is almost impossible to define. Here we are using it in a loose, but widely accepted sense to cover a group of reactions characterized by a heightened or accelerated response to a particular type of antigen, irrespective of the balance of harm, or benefit, that the altered response confers on the allergic host.

All the phenomena of hypersensitiveness could obviously be included under this general label. In the present section we are concerned with those instances in which an allergic state develops in relation to a bacterial infection.

It has already been noted that bacterial haptens may induce acute anaphylactic shock in a sensitized guinea-pig ; but before discussing allergic reactions in the wider sense we may refer briefly to the question of anaphylactic sensitization with bacterial antigens.

The available data may be summarized by stating that active sensitization has been induced with various bacterial antigens and passive sensitization with the corresponding antibodies as demonstrated by the occurrence of acute shock (Rosenau and Anderson 1907, Kraus and Doerr 1908, Holobut 1909, Kraus and Admiridzibi 1910, Zinsser and Parker 1917, Tomesik and Kurotchkin 1928, Lancefield 1928, Enders 1929, Avery and Tillet 1929), or by Dale's technique with the isolated uterus (Zinsser and Parker 1917, Zinsser and Mallory 1924, Tomesik and Kurotchkin 1928). The reactions are specific and desensitization can be demonstrated. Sensitization to bacterial antigens cannot, however, be produced with the same ease as can sensitization to such antigens as horse serum or egg white. A single injection of bacteria frequently fails to produce any demonstrable sensitization, and the most successful results have been obtained by giving repeated small injections of antigen, followed by an interval of three weeks or so before the intravenous injection of the test dose, which should be relatively large. A technical difficulty in work of this kind is introduced by the fact that many of the bacterial suspensions employed are themselves toxic when administered in any considerable dose, and the symptoms that develop in normal animals after the injection of massive doses of bacteria or bacterial products may be very similar to anaphylactic shock. The difference in the response of normal and of sensitized animals may, therefore, be relatively difficult to detect.

Turning to those instances in which specific sensitization occurs during the course of a bacterial infection—the type of hypersensitiveness with which we are most closely concerned—the classical example of an allergic response to a non-living bacterial antigen is afforded by the tuberculin reaction in its various forms.

This experimental allergic reaction is readily demonstrated in the guinea-pig. The injection into a normal guinea-pig of 2 ml. of Koch's old tuberculin—a culture of tubercle bacilli in 5 per cent. glycerine broth, grown for 6–8 weeks at 38° C., concentrated to 1/10th volume at 100° C., and freed from bacilli by filtration through a clay or kieselguhr candle—has little effect. But the injection of 0·01 ml. into a guinea-pig in the 8th or 10th week of tuberculous infection may kill it within a few hours. At necropsy, supposing the injection to have been given subcutaneously, the site of inoculation is found to be very congested, dark red or almost violet in colour. The focal glands are swollen and congested, the liver and spleen show on their surface numerous dark red spots, particularly in the neighbourhood of the multiple tuberculous lesions which are commonly present. Microscopically these red spots are seen to consist of enormously dilated capillaries. Thus the tuberculin produces a local reaction at the site of inoculation, numerous scattered focal reactions in the immediate neighbourhood of pre-existing tuberculous foci, and a rapidly fatal toxæmic effect, probably secondary to the widespread focal reactions.

This fatal generalized tuberculin reaction in the guinea-pig exemplifies the allergic response in its severest form. The development of hypersensitiveness in an infected animal may, however, be demonstrated quite as clearly by means of the localized reaction that follows the intracutaneous injection of 0·2 ml. of a high dilution (1 : 800 to 1 : 4,000) of tuberculin (see Römer and Joseph 1909, 1910, Lewis and Aronson 1923, Eagleton and Baxter 1923). For further details with regard to the tuberculin reaction, and its diagnostic use in human and veterinary medicine, see Chapter 59.

We have so far been concerned with the allergic response to a non-living antigenic material; but there is abundant evidence that the sensitized tissues respond differently from the non-sensitized to contact with living bacteria. Here again, the classical example is afforded by Koch's observations on experimental tuberculosis (Koch 1891).

When a normal guinea-pig is injected subcutaneously with living tubercle bacilli, a localized nodule appears at the site of inoculation after 10–14 days. This nodule increases in size, involves the skin, and undergoes necrosis, leaving an indurated ulcer that persists until the animal's death. Meanwhile there occurs the characteristic lymphatic spread of

infection, affecting first the regional lymphatic glands and later the more remote tissues and organs. But if living tubercle bacilli are injected into a guinea-pig that is already infected with tuberculosis of some weeks' duration the result is quite different. On the 1st or 2nd day following the reinoculation the site of injection shows an area of acute superficial inflammation 0·5–1 cm. in diameter, without the formation of any nodule in the underlying subcutaneous tissue. Next day the affected area of skin is found to be necrotic, and finally it sloughs, leaving a flat, shallow ulcer which undergoes rapid healing. The regional lymph glands are not affected; and there is no evidence of the spread of infection from the localized focus caused by the second inoculation. This rapid and energetic response does not, in this case, confer any permanent benefit on the guinea-pig, for it is destined to die from the primary infection; but it affords a striking example of a reaction that betrays at one and the same time the character of hypersensitiveness and of immunity.

Although tuberculosis happens to afford the classical illustration of allergy developing as the result of infection, there are numberless other examples. There is, indeed, little doubt that an increased sensitivity, associated with an increased vigour of response, is of frequent, perhaps of almost constant, occurrence at one stage or another of every infective disease; and it is very probable that this sensitization plays a part in pathogenesis, particularly in subacute or chronic infections.

Bacterial and Anaphylactic Hypersensitiveness and Immunity.

Among the many issues raised by the phenomena of bacterial hypersensitiveness, two may be considered as outstanding—namely, the relation between the bacterial and the anaphylactic types of hypersensitiveness, and the value of specific hypersensitiveness in defence against bacterial infection.

Zinsser (1921) was the first to distinguish the two types of hypersensitiveness. In guinea-pigs sensitized to the tubercle bacillus, he found that susceptibility to anaphylactic shock by bacillary proteins could exist independently of tuberculin skin-reactivity. The distinction was confirmed for the tubercle bacillus, and demonstrated in hypersensitiveness to pneumococci and streptococci (Mueller 1926, Enders 1929, Zinsser and Grinnell 1925, 1927, Dochez and Sherman 1925). The local skin reaction of the tuberculin type reaches its maximum slowly, after a lag of several hours, while the anaphylactic reaction takes place soon after injection of the antigen. Moreover, the tuberculin type of sensitization is best induced only after contact of the tissues with bacteria and is as a rule not induced by soluble bacterial products. Dienes (1927), however, made the interesting observation that if tuberculous guinea-pigs were sensitized with a soluble antigen like ovalbumin, the skin reactions to ovalbumin were of the delayed tuberculin type, and that this change in reactivity was most easily elicited when the ovalbumin was injected directly into a tuberculous focus. In the light of the adjuvant action of dead tubercle bacilli on the antigenicity of various substances discussed on p. 1116 it is perhaps legitimate to suggest that this result follows from an enhancement of the degree and type of antibody response, and not from a peculiarity of the antigen.

The significance of these facts is not immediately clear, but they indicate that induction of the tuberculin type of sensitivity is associated with active cellular tissue reactions in the body of the animal. The importance of the cellular element in bacterial hypersensitivity is emphasized by the work of Rich and others, who showed that whereas tissue cultures from animals in the anaphylactic hypersensitive state were unaffected by the addition of antigen, those from tuberculin-sensitive animals were killed by the addition of tuberculin (Rich and Lewis 1932, Aronson 1931, 1933, Moen and Swift 1936). Moen (1936, 1937) records a similar lethal effect with extracts of Group C streptococci on growing cultures of tissues from guinea-pigs sensitized by infection with the cocci. Rich (1941) suggests that the anaphylactic reaction depends primarily on vascular damage. When, for

example, antigen is applied to the normally non-vascular cornea of a sensitized animal, little reaction occurs; but if the cornea is vascularized by previous non-specific inflammation, the application of antigen results in a violent reaction (see Rich and Follis 1940). In bacterial hypersensitiveness, on the other hand, the reaction appears to depend on direct damage to the tissue cells.

The close association of bacterial hypersensitiveness with the tissues themselves is emphasized by the fact that passive transfer has seldom been satisfactorily achieved. In this respect, too, it differs from anaphylactic hypersensitivity, though here again, as we have seen, passive transfer is not always demonstrable.

The distinction between the two types of hypersensitiveness is of some importance in considering the rôle of the hypersensitive reactions in infection, but before proceeding to discuss that issue, it should be noted that the distinction is by no means necessarily fundamental. In the immunological sense both types are specific, but the antigenic analysis of the sensitizing substances is by no means complete, and as Bronfenbrenner (1940) points out, the peculiarities of bacterial hypersensitivity may be referable to the antigenic complexity of the bacteria and to the state of aggregation of the component antigens. The distinction is most marked with tuberculin sensitivity, but with other bacterial antigens it is less so. For instance, we have ourselves induced responses of the slowly-developing tuberculin-type in guinea-pigs sensitized by intradermal injections of the soluble antigens of *Br. melitensis*.

The rôle of hypersensitiveness in infection is a controversial matter which has centred mainly round the problem of immunity in tuberculous infection. The reader is referred to the full discussion of this subject in Chapter 59. We shall limit ourselves here to a few general observations (for an extensive review of the matter, see Rich 1941). As Rich points out, in the hypersensitive animal a bland antigenic substance behaves as though it were an acutely irritant toxin, inducing reactions of varying intensity, from mild inflammation to complete local necrosis when given locally, and fatal reactions when given systemically. *A priori*, then, we might expect that an animal hypersensitive to a bacterium would be more susceptible to infection than a normal animal, on account of the enhanced toxic action of the bacterial antigens. On the other hand, it has been observed (Chapter 53) that a tissue which is inflamed is in some circumstances capable of localizing a bacterial invasion more effectively than normal tissue.

Has the exaggerated inflammatory response to bacteria in the tissues of a hypersensitive animal any protective action? The answer to this question is complicated by the fact that a hypersensitive animal is often an immune animal, and what we infer to be a protective effect of the hypersensitive reaction may be due to an immune process independent of the sensitized state. Therefore the demonstration of a local immune response in a hypersensitive animal, even at an early stage in the process of immunization when a general immunity cannot be demonstrated (see, for example, Seastone 1939, Schlesinger and McBroom 1940, Foley 1944), is not a sure indication that the hypersensitive and the immune reactions are identical. We may circumvent this difficulty by comparing the resistance to infection of hypersensitive *and* immune animals, with that of immune desensitized animals. The validity of the comparison will depend on the degree to which our tests of desensitization—usually skin tests—really indicate desensitization of the whole animal. DeGara and Angevine (1943), for example, sensitized rabbits with killed pneumococci or streptococci, and tested the sensitivity of liver, spleen and

skin by direct injection of the corresponding bacterial suspension; there was no correlation between the degree of organ and skin reactions. But if we accept the usual criteria of desensitization, there is in some cases good evidence that hypersensitiveness does not by itself confer any immunity.

Thus Morris (1936*b*) found that injection of anti-Friedländer Type B serum into guinea-pigs rendered them both hypersensitive and immune, and that desensitization with Friedländer specific carbohydrate did not significantly reduce their immunity. Rich (1930) pointed out that the local inflammatory response to infection in the hypersensitive animal would develop after the bacteria had reached the tissues, whereas the protective effect of non-specific inflammation is manifest only when bacteria are introduced into already established lesions. If hypersensitive reactions independent of the bacterial antigens are induced in experimental infections, they appear to have little effect. Cannon and Hartley (1938), for example, injected a mixture of virulent pneumococci and ovalbumin into the skin of rabbits which were (*a*) immunized against pneumococci and sensitized to ovalbumin, (*b*) sensitized to ovalbumin, and (*c*) untreated. In groups (*b*) and (*c*) the progress of the disease was similar, ending in a septicæmic death in 48 hours; the group (*a*) animals survived. The local hypersensitive inflammatory reaction in group (*b*) animals did not affect their local resistance to the pneumococci.

It may be noted that general anaphylactic shock induced by non-bacterial antigens appears to affect the outcome of infections or intoxication either little or adversely. Weissberger (1937) reports a prolongation of experimental streptococcal bacteræmia in sensitized rabbits given a simultaneous injection of horse serum. Otto and Schafer (1937) on the other hand, found that sublethal anaphylactic shock had little influence on the course of a *Salm. enteritidis* infection of guinea-pigs that had been established for 2 days.

The allergy of infection, then, may be regarded as a state of partial immunity, as well as of increased sensitivity; and the all-inclusive classification of Doerr (1922) who would place acute anaphylaxis, natural hypersensitiveness and immunity in a single generic group, with sub-groups clearly marked by certain features but shading into each other when the focus of attention is shifted from the obvious manifestation to the underlying mechanism, offers a reasonable as well as a coherent picture of the phenomena that we have considered in this chapter.

So far as our present knowledge goes it seems very probable that anaphylaxis, hypersensitiveness, and immunity of the type dependent on specific antibodies, all result from the working of a single underlying mechanism, the character of the total reaction being determined in each particular case by a variety of secondary factors. Anaphylactic shock, for instance, is an experimental curiosity of high informative value to the student of immunity but not of any obvious significance in determining the survival or extinction of a species during the course of its evolution. The sudden introduction of a foreign antigenic substance into the circulating blood is not an event that is likely to menace any living thing, unless it is subjected to intentional human interference. It is neither paradoxical nor surprising that a mechanism, which in natural circumstances tends directly towards immunity, should react to the disadvantage of its possessor when exposed to a stimulus that formed no part of the environment in which it was evolved. Nevertheless, in accepting this picture of the essential unity of these phenomena we must not commit ourselves to a belief that the naturally occurring manifestations of allergy are necessarily beneficial or even harmless to the species of animal displaying them.

The existence of a certain biological feature in an animal is not a sufficient guarantee that the feature has necessarily contributed to the survival of the species. An adverse feature might well be preserved, provided that the intensity

of selection with respect to it was sufficiently low. The only reliable tests of biological value are those made directly on the species as it is now established in the animal world.

Infection-Immunity.

This is as convenient a place as any in which to mention a conception that is often found in current bacteriological literature—the hypothesis that there is a kind of immunity that is associated with existing, but not with past infection. In its extreme form this conception suggests that so long as an infection with a particular bacterial parasite exists, even though that infection is latent, the host will be immune to reinfection with that particular bacterium, but that once the host's tissues are freed altogether from the infecting parasite susceptibility will return.

We have already noted Koch's observation that a guinea-pig behaves differently towards a primary and a secondary infection with living tubercle bacilli. The studies of many subsequent workers have confirmed and greatly extended his findings, and have shown quite clearly that a subacute or chronic tuberculous infection is associated with a considerable resistance to reinfection.

Significant evidence bearing on the problem of infection-immunity has been obtained during the study of experimental syphilis in the rabbit. In this infection it is possible, by administering an adequate dose of neosalvarsan, or of one of the related arsenical drugs, to cure the initial infection and observe the effect of so doing on the resistance of the rabbit to reinfection.

A syphilitic keratitis may be induced in the rabbit by the injection of *Trep. pallidum* into the anterior chamber of the eye (see Bertarelli 1906, 1907, 1908), a chancre may be produced by injection into the skin of the scrotum (Tomasczewski 1910), and a characteristic lesion follows injection into the testicle (Parodi 1907).

When an infected rabbit is reinoculated with the same strain of *Trep. pallidum* after a relatively short interval a local chancre does not develop (Truffi 1910); but reinfection with a different strain may induce a typical local lesion (Kolle and Schlossberger 1926). Brown and Pearce (1921) infected a number of rabbits and treated some of them with arsphenamine shortly after the development of the primary chancre. Five days later these rabbits were injected with the same strain of *Trep. pallidum*. Most of the treated animals developed chancres. The untreated animals did not.

Kolle (1922, 1924) treated rabbits with neosalvarsan at various stages of infection. He found that rabbits treated within about 6 weeks after the initial infection usually responded to a subsequent injection by developing a new chancre. When treatment was delayed beyond this point, reinfection seldom gave rise to a chancre. When treatment was delayed for about 3 months a new chancre could very rarely be produced. Rabbits that were treated and reinfected at long intervals after the primary infection (75–250 days) were not, however, completely resistant to reinfection (see Kolle and Prigge 1927, 1929). Tissue extracts from treated rabbits that had not been reinfected were non-infective for normal rabbits, but similar extracts from treated and reinfected rabbits gave rise to characteristic lesions, showing that the spirochætes had gained access to the tissues and were presumably multiplying in them, at least to a limited extent. On these grounds Kolle and his colleagues maintain that the immunity to reinfectioin dispayed by rabbits that have been treated in this way is only a " chancre immunity." The

exact significance of this term is a little dubious. We have noted many examples of a partial antibacterial immunity in which bacteria may persist for long periods in the tissues without giving rise to any of the lesions that they produce in non-immunized animals. Moreover, the evidence suggests that a more complete immunity against *Trep. pallidum* may be induced under optimal conditions. Uhlenhuth and Grossmann (1928) treated syphilitic rabbits with neosalvarsan 107 to 506 days after the primary infection. Attempted reinfection 3 to 10 months after treatment gave rise to no obvious lesions; and in only 4 of 11 rabbits could spirochætes be demonstrated in the tissues. (For a more detailed discussion and further references see Chapter 81.)

In summary, we may say that immunity to reinfection appears to be established within a short time after the appearance of the initial lesion. At this stage the maintenance of immunity depends on the maintenance of infection; complete chemotherapeutic cure is followed by a return of susceptibility. But after a longer period of infection a partial immunity is established that may persist after complete chemotherapeutic cure; and, after still longer periods of infection, a complete immunity may be established that is independent of the persistence of infection.

Whether it is necessary to assume the existence of some mechanism other than those we have considered in this and in preceding chapters, in order to account for the immunity to reinfection— or, as it is more appropriately termed, to " superinfection "—during the early stages of the initial infection, is a question that cannot yet be answered. We may, at least, note that this resistance to superinfection appears to be type-specific. As regards the resistance developed during the later stages of infection, it seems highly probable that it can be accounted for by a slow acquirement of an immunity of the usual antibacterial type.

SUMMARY

(1) Acute anaphylactic shock may be produced by inoculating into an animal any antigen to which it has previously been sensitized. The symptoms and lesions associated with shock in any particular case are determined by the anatomical and functional peculiarities of the species of animal employed, not by the character of the antigenic material injected.

(2) Sensitization is specific. It may be induced either actively or passively, and hence must depend, at least in part, on some form of antigen-antibody reaction.

(3) Active sensitization is induced—in the case of such an animal as the guinea-pig and such an antigen as horse serum—by a single minimal injection of antigen. After this injection there follows a latent period during which hypersensitiveness develops, and during this period no further injections of antigen must be given. Once the anaphylactic state has been actively induced it lasts for months or years.

(4) Passive sensitization is induced by the injection into a normal animal of the serum from a sensitized or from an immunized animal—that is by any serum containing the specific antibody. There is a correlation between the antibody content of a serum and its effectiveness as a sensitizing agent; and for this reason the serum of an immune animal is more effective (*i.e.*, sensitizes in smaller doses) than the serum of a hypersensitive animal. The anaphylactic state that is induced by the injection of an antibody-containing serum takes some hours to develop. To ensure typical shock the shocking dose of antigen should not be injected until

8 to 24 hours have elapsed after the injection of the antiserum. During this latent period the antibodies have, for the most part, disappeared from the blood and become fixed on or in the tissue cells. The anaphylactic state when passively induced passes off in a few days or weeks.

(5) To produce typical shock in either an actively or passively sensitized animal, the shocking dose of antigen must be introduced into the blood stream rapidly and in adequate, though not necessarily in large amount. Intravenous injection gives the most consistent results, and in many cases is the only route by which typical shock can be induced.

(6) The injection of haptens, or partial antigens, fails to induce active sensitization, just as it fails to induce active immunity. Complex haptens, such as the bacterial polysaccharides, will induce acute shock when injected into an animal actively sensitized by the injection of the corresponding full antigen, or passively sensitized by the injection of a specific antiserum. Less complex haptens will neither induce active sensitization nor induce shock in a sensitized animal; but when injected into such an animal they will inhibit the induction of shock by the corresponding antigen. In a sensitized animal antigens and haptens behave *in vivo* as they behave in the precipitin reaction *in vitro*. Certain simple, highly reactive organic compounds are capable of inducing hypersensitiveness, by combining with and modifying the proteins of the body to form antigenic complexes.

(7) The sensitized animal may be desensitized by the injection of the specific antigen in amounts too small to induce acute shock or by a route that ensures slow absorption so that there is no rapid transport to the circulating blood.

(8) The anaphylactic state can be demonstrated in an isolated tissue or organ as well as in the animal as a whole; thus, the uterus of a sensitized guinea-pig reacts *in vitro* to contact with the antigen employed in sensitization.

(9) The balance of experimental evidence is strongly in favour of the view that the anaphylactic response is the result of an antigen-antibody reaction occurring on or in the cells that have removed the antibody from the blood and in some way fixed it themselves. The anaphylactic state is associated with the presence of fixed antibody and the absence of circulating antibody, though in the rabbit, and occasionally in other animals, the white cells of the blood themselves are sensitive to shock. The immune state is associated with the presence of circulating antibody in a concentration sufficient to protect the fixed antibody that is also present.

The difference between the anaphylactic and immune state is quantitative rather than qualitative, depending upon the balance between circulating and fixed antibody.

(10) Many of the most dramatic features of the syndrome of anaphylactic shock are the result not of the antigen-antibody reaction itself but of the liberation, among other substances, of histamine by the injured cells, and the secondary response of the histamine-sensitive cells throughout the body.

(11) A great variety of substances, when injected intravenously, may give rise to a syndrome resembling, in many but not in all particulars, that of anaphylactic shock. These reactions are spoken of as anaphylactoid reactions. The resemblance is not due to any similarity in the underlying mechanism, but to the fact that, in each case, cellular injury is followed by the liberation of histamine and other substances, and this by the characteristic syndrome of histamine shock.

(12) Apart from acute anaphylactic shock there are other examples of hypersensitiveness, among them the natural idiosyncrasies that have been observed in

man to contact with particular materials, such as pollens, horse serum, horse dander, and certain drugs.

(13) In spite of obvious superficial differences between this condition and experimental anaphylaxis in the guinea-pig, the knowledge that has been gained in recent years—and particularly the demonstration that skin sensitivity to these atopic substances can be passively transferred—has lent support to the view that the underlying mechanism is essentially the same.

(14) Specific hypersensitiveness may also develop in the course of natural or experimental bacterial infection. This type of hypersensitiveness—which is sometimes referred to as *allergy*—is characterized by an accelerated and exaggerated reaction to contact with the infecting bacterium or its products. It partakes of the character of hypersensitiveness in these respects. It partakes of the character of immunity in that the allergic reaction is often associated with the localization of infection. This type of reaction may sometimes be demonstrated in animals that have been immunized by the injection of bacteria or bacterial products, as well as in animals that are suffering from an active or quiescent infection. It may be an important factor in the development of natural or artificial immunity.

(15) Acute anaphylaxis, natural hypersensitiveness, the allergic response and specific antibacterial immunity would appear to depend on the same fundamental mechanism—the union of an antigen with its specific antibody. The differences between them depend on the nature of the antigen concerned (living parasite, toxic substance or intrinsically inert material), the nature of the animal into whose tissues it penetrates, the rate and route of penetration, the distribution of the antibody in the animal's tissues and body fluids, the secondary effects of the primary cellular reactions, and a variety of other factors.

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CHAPTER 52

CERTAIN NON-SPECIFIC MECHANISMS IN GENERAL IMMUNITY

THE integrated protective mechanisms which we have described in the preceding chapters are specific in their action. When fully established, by nature or by art, they increase the resistance of the body against some particular bacterial infection, not against bacterial infections in general. In the present chapter we shall consider certain mechanisms from which this element of specificity is absent.

β-Lysins, Leukins and Plakins.—Among the earliest records of experimental immunity are a series of observations on anthrax infection, which establish clearly the presence in the serum of certain animal species of bactericidal substances different in kind from the bactericidal complex formed of specific sensitizer and complement. Thus the serum of the rat is actively bactericidal for the anthrax bacillus, and this activity remains after heating the serum at a temperature of 56–60° C. for 30–40 minutes (von Behring 1889, 1892, Malvoz 1902, Pirenne 1904). The name *β-lysins* has since been applied to these relatively thermostable bactericidal serum constituents.

Another series of observations, dating from the same early period, have shown that similar thermostable bactericidal substances can be extracted from suspensions of polymorphonuclear leucocytes. Like the β-lysins these *leukins* are restricted in their range of activity, some bacterial species, in particular the spore-bearing aerobes such as *B. anthracis* and *B. subtilis*, being highly sensitive to their action, other species being relatively or entirely resistant. There is some divergence of evidence with regard to the susceptibility or insusceptibility of different bacteria, and it seems possible that the leukins derived from one animal species may differ in this respect from those derived from another. Nor can it be said that there is yet any certainty as to whether the β-lysins and leukins are relatively simple substances, or complex systems like serum complement, with its end-piece, mid-piece and third and fourth components. Pettersson (1936) distinguishes two components of β-lysin action, the β-lysin and an "activating" substance. Unlike the specific α-lysins, which are absorbed by bacteria and "activated" by complement, the "activating" substances of the β-lysin, not the thermostable β-lysin, are absorbed by susceptible bacteria. (See Hahn 1895, Schattenfroh 1897, 1899. Pettersson 1906, 1908*a*, *b*, Hiss 1908, Hiss and Zinsser 1908, Zinsser and Hiss 1908, Schneider 1909, Zinsser 1910, Kling 1910, Weil 1911, Blum 1922, Haussmann 1925, Mackie and Finkelstein 1932, Mackie *et al.* 1932, Gordon and Wood 1937, Hjorth 1937, Gratia and Goreckzy 1938, Oldfelt 1942.)

There are observations which suggest that substances with a general similarity to leukins may be extracted from blood platelets (see Pettersson, above, Gruber and Futaki 1907, Barreau 1909), and these have been called *plakins*. It is not certain what relation the β-lysins bear to the leukins or to the plakins. There are apparent

differences in thermostability—the β-lysins are inactivated at temperatures between 65° and 70° C., the leukins not below 75° C. But these differences have hardly been established with sufficient sharpness, or with sufficient regard to possible effects of differences in the containing medium, to be accepted as a clear differential criterion. It seems certain, however, that many animal species may contain no demonstrable β-lysins in their serum, although leukins may readily be derived from their leucocytes.

Our present knowledge may be summarized by the statement that there exist in the normal sera of certain animal species thermostable bactericidal substances which are non-specific in their action, in the sense in which immunological specificity is generally understood—though their range of activity is limited by the varying sensitiveness of different bacterial species—and which are not increased in amount as the result of immunization. Bactericidal substances having very similar properties may be extracted from leucocytes and perhaps from blood platelets.

The relation of these substances to effective immunity is by no means clear. To take the case of anthrax, the relatively resistant rat has β-lysins in its serum, so has the susceptible rabbit, while the relatively resistant dog has none (see Ledingham 1922).

The significance of the leukins and plakins is still more difficult to assess. They are not identical with leucocytic enzymes (see Fleischmann 1928) though the participation of extracellular enzymes of this kind in leukin action cannot be excluded. That the leukins—using that term for the moment as a generic name for the antibacterial substances present in or derived from leucocytes—play some part in resistance to infection, seems a highly probable assumption; but we do not know whether their rôle is primary or secondary, nor how it is related to the defence mechanisms that we have considered in more detail above.

We may note, in this connection, an observation by Buxton and Torrey (1906) which suggests that the polymorphonuclear cells play a significant rôle as bactericidal agents, even when they are not concerned in the primary phagocytic reaction. The injection of typhoid bacilli into the peritoneal cavity of the rabbit is followed by the rapid deposition of a large proportion of the injected bacteria on the surface of the omentum. Here they are in part destroyed by extracellular lysis, in part ingested by the omental histiocytes. During the first 4–6 hours the polymorphonuclear cells play little if any part in the events on the omental surface, but after that interval there is a marked and increasing polymorphonuclear reaction which varies in intensity in different animals, and the fate of the rabbit appears to depend in part on the successful mobilization of the polymorphonuclear cells. When they collect rapidly and in large numbers the clearance of typhoid bacilli is complete, and the animal recovers. When they are scanty or slow in arrival secondary centres of bacterial multiplication appear, and the animal dies with a generalized bacteræmic infection. These observations have not been repeated in detail by later observers, and are not therefore established on a sufficiently broad basis to allow of any general induction; but they are clearly suggestive.

Variations in the Phagocytic Power of the Leucocytes.—We have seen that one animal differs from another in the phagocytic power of its whole blood and that this difference is determined, at least in part, by the presence or relative concentration of sensitizing antibodies, the opsonins or bacteriotropins, whose action is increased in effectiveness by the normal serum complement. This opsonizing action is specific, so far as the sensitizing antibody is concerned, and may be increased

or induced *de novo* as the result of specific immunization. But the phagocytic activity of the leucocytes, as was first shown clearly by Shattock and Dudgeon (1908), is itself variable. Leucocytes from different persons, and particularly from persons suffering from various infections, show a significant difference in their ability to ingest not only various bacteria, but also inert particles, such as melanin. The latter observation is of importance, since it indicates that such variations in phagocytic activity are independent of the nature of the phagocytable object (see also Glynn and Cox 1909–10, Boughton 1910, Rosenow 1910, Hektoen 1911, Wright 1931).

Lysozyme.—In Chapter 45 we referred to the bactericidal substance described by Fleming under the name of lysozyme and noted that, in high concentration, it has some bactericidal effect on pathogenic bacteria as well as on those non-pathogenic organisms that are particularly susceptible to its action. What part this substance plays in the defence mechanisms of the tissues it is, at the moment, impossible to say.

Induced Variations in the Non-specific Bactericidal Power of the Blood.—This leads us to a consideration, necessarily brief, of the possibility of inducing an effective immunity by increasing the non-specific bactericidal mechanisms of the cells and fluids of the body. Wright and his collaborators (see Wright, Colebrook and Storer 1923, Wright 1931, 1942) have been active in exploring this field. Using specially devised *in vitro* methods of testing the bactericidal power of the whole blood, they have found that the addition of living or killed bacterial suspensions to blood in the test tube, or the injection of similar suspensions into the living animal, results in a very rapid increase in bactericidal power. This increase they attribute, in greater part, to the liberation by the leucocytes of directly bactericidal substances, in lesser part to increased opsonic power. Although this response is not specific—in the sense that there need be no antigenic relationship between the bacterium employed to induce the response and the bacterium against which the bactericidal effect is exerted—some bacterial suspensions have been found to provide a more effective stimulus than others.

This increased bactericidal power is developed with great rapidity—within a few minutes or hours—and is relatively transient, lasting at most for a period of days. It may be induced by a wide variety of stimuli.

Thus Prausnitz and Meissner (1925) record an increase in the bactericidal power of the whole blood after the injection of yatren and aolan. Fleming (1926) records a characteristic response after the injection of salt solutions into rabbits, or after the injection of nuclein (1928). Colebrook, Eidenow and Hill (1924) have recorded a similar response after exposure to ultra-violet light or to dark heat, and also after the application of a mustard plaster (see also Wenger 1938).

In some instances, at least, this temporary increase in the bactericidal power of the blood would seem to be due to, or associated with, an increase in the opsonic power of the serum (see Ledingham and Bulloch 1906, Bedson 1915). Thus, Bedson found that an injection of nuclein was followed by an increase in the normal opsonic action, which was detectable within 20–30 minutes, was still demonstrable after 24 hours, but then rapidly faded.

It must be emphasized that the *in vitro* mixtures of blood and bacteria employed in these investigations are exceedingly complex, and may be susceptible to a number of unrecognized environmental factors that are not operative *in vivo*. The point is well illustrated in the careful study of Fuller, Colebrook and Maxted (1939), who found that

the bactericidal power of human blood, though not of rabbit or mouse blood, was almost completely suppressed if CO_2 was allowed to accumulate in the tubes containing the blood-bacterium mixture under study. (See also Tillett 1937, Tillett and Stock 1937, Miles and Misra 1938.)

The value of this reaction as a protective mechanism is exceedingly difficult to determine. It may be concerned in some, at least, of those instances in which a temporary, or trivial, increase in resistance has been observed to follow a procedure which could not have resulted in the production of specific antibodies.

H. D. Wright, for example, has noted that rabbits may show an increased resistance to the intravenous injection of virulent pneumococci within 5 hours of the injection of a killed suspension of the same organism, a time interval too short to permit of antibody production. Similarly, Teague and McWilliams (1917) report that the intravenous injection of killed typhoid bacilli into rabbits is followed, within 24 hours, by a slight resistance to the injection of living bacilli. In other instances, the injection of a killed suspension of a given bacterium has been observed to induce a slight, or transient, increase in resistance to some antigenically unrelated organism (H. D. Wright 1927, Nukada and Arifuku 1931, and many others). The triviality, or transitoriness, of this non-specific increase in resistance should be stressed ; and in this connection we may recall the effect of non-specific immunization in protecting mice against natural infection with *Salm. typhi-murium* (see Table 73, p. 1056). The limited mean survival time of control unvaccinated mice, in an infected herd, was $26{\cdot}26 \pm 0{\cdot}641$ days, that of mice vaccinated with *Staphylococcus anreus* (an entirely unrelated organism) was $29{\cdot}86 \pm 0{\cdot}714$ days. Mice immunized with a specific vaccine lived on the average $35{\cdot}31 \pm 0{\cdot}884$ days. The difference between the non-specifically immunized and the control mice was trivial, barely attaining statistical significance, but two other non-specific vaccines gave a closely similar result, while a third, not included in Table 73, gave no protection at all.

Cowan (1939) immunized rabbits with *Past. pseudotuberculosis* and found that the rate of disappearance from the blood of intravenously injected *Staph. aureus*, both immediately after injection and after establishment of the secondary bacteræmia (see p. 1047) was greater than that in normal animals, and equal to that in rabbits immunized with *Staph. aureus* vaccines. In view of the complete antigenic dissimilarity of the pasteurella and the staphylococcus, this effect is clearly non-specific. In this connection we may note that Day (1942, 1944) reports an increased immunity of mice to pneumococcal infection by inoculation with extracts of a wide variety of cocci and bacilli. Day, however, regards the immunity as induced by a common antigen. We also find in naturally occurring disease instances of apparently non-specific stimulation of antibacterial mechanisms which may be analogous to the phenomena discussed above. Thus Wulff (1934), Tillett (1937) and Hjorth (1937) describe a marked increase in the bactericidal properties of serum that occurs in man during fever, decreasing to normal with convalescence from the pyrexial disease. According to Hjorth, the effect is due to substances resembling β-lysins. A similar substance may be responsible for the marked anti-staphylococcal activity of tuberculous pleural effusions recorded by Tobler and Pinner (1943).

Taking the evidence as a whole, it would seem that the increase in resistance afforded by non-specific stimulants of this class are for the most too slight, too transient, or both, to play any significant part in prophylaxis against infection. Whether they can be exploited therapeutically is a different problem.

The Shwartzman Phenomenon.

In the preceding paragraphs we have considered various reactions that are associated with a non-specific increase in resistance. In concluding this chapter it will be convenient to refer briefly to an experimental procedure that is associated

with a localized and non-specific increase in sensitivity. Shwartzman (1928, 1929*a*, *b*, 1931, 1932, 1933) has described a curious phenomenon that has become known by his name, and has been studied in considerable detail by a number of other workers (see Gratia and Linz 1931, 1932, Gross 1931, Peck and Sobotka 1931, Gratia, 1932, Peck 1933, 1934, Freund 1934*a*, *b*, Freund and Smith 1934, Freund and Hosmer 1935, Gerber 1936, Ogata 1936, Shwartzman 1936*a*, *b*, Witebsky and Neter 1936, Giuffré 1937, Shwartzman *et al.* 1937, 1938, Morell and Shwartzman 1938, Alechinsky 1938, 1939, Weir 1938, Ayo 1943).

If a rabbit is injected intradermally with a small amount of a filtrate of a culture of *Salm. typhi*, and 24 hours later is injected intravenously with the same filtrate, the intravenous injection is followed, within a few hours, by the development of a hæmorrhagic lesion at the site of the intradermal injection. It is not, however, necessary that the same organism should be used for the intradermal injection and for the subsequent intravenous injection, or that the organisms used for the two injections should be antigenically related. Filtrates from, or suspensions of, a variety of bacteria will sensitize the skin to the intravenous injection of an equally wide variety of bacteria or bacterial filtrates. Not all bacteria contain effective sensitizing substances, nor is the local sensitizing effect the result of a simple inflammatory reaction. Thus (Shwartzman 1928), the injection of uninoculated culture media, of turpentine or of filtrates of various strains of streptococci, failed to sensitize the skin to the subsequent intravenous injection of filtrates of typhoid cultures. The sensitizing or, as Shwartzman prefers to call them, the " preparatory " substances are thermostable, antigenic, of relatively large particle size and often closely associated if not identical with the " complete antigens " or endotoxins of coliform and salmonella bacilli (see, for example, Iványovics 1936). Purified preparatory substances are said to produce no detectable change in the tissues. Not all Gram-negative bacteria contain preparatory substances (see Witebsky and Salm 1937, Wise and Kerby 1943). Nevertheless, the vascular endothelium is in some way altered by the preparations so that the intravenous " provoking " dose causes severe hæmorrhage and necrosis in the prepared site. The range of provocative substances is much wider than the range of preparatory substances, and includes starch, agar, preparations of bacteria that are incapable of " preparing " the tissues, and animal sera. If an animal is anaphylactically sensitive to the preparatory substance, the preparatory effect is enhanced (Angevine and DeGara 1941); if it is anaphylactically sensitive to the provoking substance, the Shwartzman reaction is enhanced; but the Shwartzman reaction is neither anaphylactic nor is it related to the formation in sera of the toxic substances discussed in Chapter 51 under anaphylatoxins (Shwartzman 1937). Organs other than the skin, particularly the kidney, can be " prepared " by Shwartzman substances, and a similar though general hæmorrhagic reaction can be induced in rabbits by an intravenous preparation followed by an intravenous provocative dose (Sanarelli 1924; see Alechinsky 1939). It is of some interest that young animals are relatively refractory, both as regards preparation and provocation.

Cohen and Moolten (1943) record a number of experiments on the rôle of preparatory factor and provocative factor in experimental infections of rabbits with a strain of *F. necrophorus*. A virulent strain yielded both factors, and an avirulent strain only the preparatory factor. In rabbits infected intratracheally with the avirulent strain, fatal infection could be induced by the intravenous injection, 24 hours later, of provocative factor derived either from the virulent strain, or from an unrelated organism like the meningococcus. Though the results clearly suggest that the two factors may be concerned in the genesis of an infective lesion, it is not clear in what way pathogenesis is related to phenomena elicited, as the Shwartzman reaction is, by the arbitrary intravenous injection of a bacterial fraction 24 hours after the onset of infection.

The possibility that in natural infections there may be local alterations in the reactivity of tissues, particularly of vascular tissues, induced non-specifically by substances of bacterial

origin, cannot be overlooked in our attempts to analyse the phenomena of resistance to infection. But aside from a possible connection between hæmorrhagic phenomena in certain chronic infections in man (see Shwartzman, Klemperer and Gerber 1936) it is at present impossible to say what part this mechanism plays in natural infection and resistance, or in artificial immunization.

SUMMARY

(1) In addition to the specific factors that determine antibacterial immunity, there are certain non-specific factors which appear to play some part in the reaction of the tissues to invading bacteria. The exact significance of these factors, and their relation to the general defence mechanism, are at the moment doubtful.

(2) Among these factors are certain thermostable bacteriolysins—the β-lysins—which are present in the sera of certain animal species. Their bactericidal action is limited to particular bacterial species, and it seems doubtful whether they confer an effective immunity even against these. They are not increased in amount as the result of immunization though they may be increased by non-specific stimuli.

(3) Bactericidal substances may be extracted from leucocytes (the leukins) and perhaps from blood platelets (the plakins). These substances bear many resemblances to the β-lysins, and appear to be even more thermostable. They probably play some part in antibacterial immunity, but what that part is remains, at the moment, an unsolved problem.

(4) Leucocytes vary in their phagocytic activity, and it seems likely that such variations may be induced in response to infection, and perhaps to immunization.

(5) Various non-specific stimuli are capable of inducing a rapid but transient increase in the bactericidal power of the blood, probably determined by some of the factors referred to above. The resulting increase in resistance is too slight, and too temporary, to be of practical prophylactic value. Non-specific stimuli are in some circumstances able to increase the resistance of the animal as a whole, but the increase is small compared with that induced by specific immunization.

(6) The phenomenon described by Shwartzman provides an example of non-specific sensitization.

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CHAPTER 53

LOCAL IMMUNITY

THE defence mechanisms with which we have hitherto been concerned have for the most part been generally effective throughout the tissues of the host; and by whatever route bacteria or their products gain access to the tissues these mechanisms will tend to protect the body as a whole.

Another type of immunity may be conceived, confined to one particular area of the body, or to one particular kind of tissue. In Chapter 45 we have discussed certain mechanisms that hinder the access of bacteria to the tissues, many of which, like the lysozyme in the conjunctival sac, or the acidity of the gastric juice, are functions of a particular locality in the body. We may distinguish these from the mechanisms of local immunity which we consider in this chapter, by assuming that they act on the infecting organism before it actually invades the tissues. It will be clear, nevertheless, that the distinction, though convenient, is arbitrary, for the hindering mechanisms may in fact be located in the tissues; and even if their action, like that of gastric juice, takes place manifestly outside the tissues, the effect is ultimately dependent on local tissue function. We must, therefore, be prepared if necessary to accept an "external" hindering mechanism as an expression of a true local immunity, though we shall in the meantime confine ourselves to manifestations of resistance within the tissues.

A heightened local resistance of this kind may or may not be associated with a significant increase in the resistance of the body as a whole; moreover, it may depend on a local variation in the response of a generalized defence mechanism; or upon the reaction of special tissues or types of cell in the area under consideration. There has been a tendency to confuse the issue of local versus general immunity by an inadequate definition of the problem to be solved. The concept of a local immunity as such has been imperfectly separated from hypotheses regarding the mechanisms on which it depends. In some cases the term has been used as though it implied a tissue or cellular immunity, not conditioned by humoral factors. In others there has been a failure to distinguish clearly between specific and non-specific effects. It will simplify discussion if we consider the available evidence from each point of view in turn.

What evidence is there for the existence of a local as opposed to a general immunity?

As long ago as 1887 Meierowitsch (1888) noted that rabbits which had recovered from an experimental attack of erysipelas were resistant to subsequent inoculations of the causative streptococcus during periods up to 2 months. This observation was confirmed by many later workers, and Gromakowsky (1895) noted that animals

which had become resistant to a local cutaneous inoculation did not always withstand an intraperitoneal inoculation.

This phenomenon was studied in much greater detail by Cobbett and Melsome (1895, 1898). They concluded, on the basis of a considerable series of experiments, that an attack of erysipelas in the rabbit completely protects the area of skin affected against a subsequent inoculation of the specific streptococcus; ; while on the rest of the body it confers an immunity which is usually less marked and is sometimes entirely absent. They noted also that, when streptococci were inoculated into the skin of a rabbit's ear after the subsidence in that ear of an attack of erysipelas, an inflammatory reaction quickly appeared but subsided before the typical erysipelatous lesion had made any appreciable progress in a control rabbit. This rapid but transitory inflammatory reaction was most marked in ears that had been locally immunized, less marked in ears that shared in a general protection. Gay and Rhodes (1922) concluded from a study of the same problem that a rabbit which had recovered from an attack of erysipelas was completely protected against subsequent intracutaneous injections of the causative streptococcus, irrespective of its exact locality, for a period of at least 3 months. They were unable to obtain a similar immunity by the intracutaneous injection of cultures killed by heat or by alcohol. Immunity against intracutaneous inoculation was not associated with immunity against intravenous inoculation ; while immunization by the intravenous route was found to afford more protection against subsequent intravenous inoculation than against intracutaneous inoculation. Rivers (1925) carried out a series of experiments in which rabbits were given repeated intracutaneous injections of living hæmolytic streptococci derived from a case of erysipelas. He noted that the reactions following successive injections showed a progressive decrease in severity. After several such injections the sera of the rabbits were tested for the presence of protective antibodies. The serum to be tested was mixed with a suspension of living streptococci and injected into the skin of a normal rabbit ; at another site in the same animal another injection was made consisting of a mixture of the same streptococcal suspension and normal serum. The sera from the immunized rabbits had a marked protective action, normal serum had none. The studies of Amoss and Bliss (1927) bring out further points of interest. Like other observers they found that an area of skin which had been involved in an erysipelatous reaction was definitely more resistant to a subsequent injection of the causative streptococcus, and that if the injections were repeated the degree of resistance progressively increased. They noted that the primary lesion in the skin of the rabbit's flank tended to spread ventrally from the site of inoculation in the direction of the lymph flow ; and they found that when the area involved in the primary reaction and the skin in its immediate neighbourhood were tested by subsequent intradermal inoculations the area of increased resistance extended ventrally beyond the area of primary reaction, but not anteriorly, posteriorly or dorsally. At this period no increased resistance could be demonstrated in the skin of the opposite flank. After prolonged immunization the skin at a distance from the site of inoculation might show increased resistance, but this generalized skin immunity was associated with a humoral immunity, evidenced by the appearance of specific antibodies in the blood.

Taking these observations as a whole it would appear that a local immunity to hæmolytic streptococci may exist in the absence of a general immunity, and that, when repeated local injections lead to a general immunizing response, the level of immunity may, for a time at least, be higher in the immediate neighbourhood of the site of inoculation than in the tissues generally. It may be noted that the severe inflammatory reaction associated with an attack of erysipelas is more effective in inducing a local immunity than is the relatively trivial reaction that follows the injection of a killed suspension of streptococci ; and that there is evidence that the tissues become allergic during the immunizing infection.

Although experimental erysipelas provides the best-documented example of a local as opposed to a general immunity, many analogous observations have been recorded in relation to other infections. Cooper (1926), for instance, states that rabbits may be immunized against the injection of virulent pneumococci into the buccal mucosa by repeated injections, into the same tissue, of a killed pneumococcal vaccine, but not by injections of the same vaccine given subcutaneously or intradermally. Other examples of a similar kind will be noted in the succeeding paragraphs.

There are also many experimental observations in relation to virus diseases (see Chapter 55) which suggest that a local immunity plays an important part in determining the reaction of the body as a whole.

We need not doubt that a local immunity exists, in the sense that one area of the tissues is more resistant than another to the introduction into it of a living bacterium or virus. The problem at issue is whether these localized differences in resistance can be accounted for by local variations in the effectiveness of the mechanisms we have considered in previous chapters, or whether some essentially different mechanism is involved.

Is the distribution of a local immunity confined to a particular type of cell or tissue affecting that type of cell or tissue wherever it occurs in the body, or is this distribution determined by the site of inoculation, affecting all cells or tissues in the immediate neighbourhood of that site?

We may particularize our question in regard to the phenomena considered in the preceding section by inquiring whether it is the skin, as such and apart from other tissues, that is rendered resistant by the intracutaneous injection of living hæmolytic streptococci, or whether the effect is limited to the area of the initial erysipelatous reaction, affecting in that area all the cells or tissues involved in the inflammatory process.

The evidence is somewhat conflicting. The observations of Gay and Rhodes appear to favour the view that the skin as such irrespective of its nearness or remoteness to the site of the primary inflammatory reaction is rendered resistant by a localized experimental erysipelas. The findings of most other workers suggest that the distribution of resistance is, in the first instance, confined to the area affected by the erysipelatous reaction, though other areas of skin share in the more general immunity that may be induced by repeated injections of streptococci. It should be noted, as Amoss and Bliss showed, that outside the area of erysipelatous reaction, the skin showing the greatest increase in immunity was that lying in the path of drainage through the underlying lymphatic plexus. The point is of some importance in deciding whether the increased immunity displayed by the skin as a whole results from immunization of the skin as an organ, or is only an especially marked manifestation of a general immunity.

Landsteiner and Chase (1939) record some experiments which are relevant to the issue. They were able to sensitize the entire skin of a guinea-pig to poison ivy by local application of an extract to one area of it. Isolation of the area of application, by removing an annular piece of skin so as to leave an island, did not impair sensitization even though the whole thickness of the skin was thus removed. When the underlying lymphatic plexus, and consequently the only pathway for the ready absorption of the extract into the body as a whole was severed, general sensitization did not follow application of the extract.

The question posed leads naturally to the one that follows it, and data relevant to its solution will be considered under this and other heads.

Does the local immunity as such, and apart from an associated humoral immunity, confer an increased resistance on the body as a whole?

It will be convenient to commence our discussion of this question by setting out, in their baldest terms, the views that have been put forward by Besredka (1919, 1920, 1921).

He notes that many, if not all, pathogenic bacteria show a marked tendency to specific localization in particular tissues of the body. Thus the typhoid, paratyphoid and dysentery bacilli cause intestinal infection, while the anthrax bacillus commonly causes infection of the skin. He would, however, carry this argument much further than those who regard such frequency distributions as the expression of a modal type of infection, determined by a variety of interacting factors and often departed from in particular instances. He believes, for instance, that the localization is in no way dependent on the route by which the bacteria enter the body. By whatever path typhoid, paratyphoid or dysentery bacilli gain access to the tissues they pass to the intestines, and there produce their distinctive lesions. Similarly, for a fatal anthrax infection to result, the anthrax bacillus, however introduced, must reach the skin.

As a logical corollary, a naturally immune or an actively immunized animal owes its increased resistance to the local immunity possessed by the particular tissues which are, in susceptible animals, specifically liable to attack. If these susceptible tissues are rendered immune then the whole animal is immune, because the specific localization which is an essential feature of bacterial infection can no longer occur. Thus, in the last analysis, general immunity is simply the expression of effective local immunity. The various antigen-antibody reactions that have been observed are, in reality, of altogether minor importance.

These conclusions are clearly revolutionary and provocative ; and like all provocative conclusions based on experimental study they have acted as a stimulus to further research. Taken as they stand, it seems hardly necessary to do more than confront them with the evidence set out in the preceding chapters in order to demonstrate that they cannot be accepted as an adequate picture of the facts. Whether a localized immunity in Besredka's sense plays any part in resistance to bacterial infection is another matter.

Taking the case of experimental typhoid, paratyphoid or dysentery infection in rabbits, Besredka lays emphasis on the point that these animals can be effectively immunized by feeding with dead or living cultures. When dead bacterial cells are used for immunization, it is sometimes necessary to administer in addition a small dose of bile, which causes minimal lesions of the mucous membrane. The fact that an increased resistance can be induced in this way does not, however, afford any evidence in favour of the conception of a specific induced immunity of the intestinal mucosa. We have seen (p. 1117) that the administration of dead or living bacteria by the mouth is often followed by the passage of bacterial antigens from the intestines to the tissues and this, in its turn, by the usual antibody-forming response. The argument on which Besredka lays most stress in this particular instance is the following. He states that when dysentery bacilli are administered to rabbits by the mouth agglutinins may appear in the blood after the first administration, but they soon disappear, and do not reappear after any subsequent administration. He records analogous observations in the case of rabbits immunized by the oral administration of typhoid bacilli after preliminary treatment with bile ; and, in both cases, he interprets the early disappearance of agglutinins, their failure to reappear after subsequent administration of bacteria *per os*, and the increasing resistance to infection by any route, to the establishment of a local immunity of the intestinal mucosa, which presents an impenetrable barrier to the passage of bacteria from the intestines to the tissues, and at the same time

prevents the essential selective localization of the bacilli should they gain access to the tissues by some other route. The few experiments recorded by Besredka seem quite inadequate to form a basis for such an important and far-reaching conclusion; and, as Otten and Kirschner (1927) have pointed out, the crucial observation of the disappearance of antibodies from the blood during a series of repeated administrations of vaccine *per os* finds no adequate confirmation in the results obtained by other workers.

Turning to the case of anthrax, controversy has centred round Besredka's contention that the skin alone is susceptible to this infection. Balteano (1922) states that he has confirmed Besredka's findings. Plotz (1924) carried out a series of experiments in which cultures of *B. anthracis* were enclosed in thin-walled glass bottles and introduced into the subcutaneous tissue of rabbits. The wounds were allowed to heal and at various periods thereafter the bulbs were broken *in situ* and the contained culture was allowed to escape into the surrounding tissues. The majority of such animals survived, and Plotz concludes that the rabbit can resist a large subcutaneous inoculation of *B. anthracis*, provided that the bacilli do not come into contact with any wound of the skin.

Other investigators have, however, recorded quite different results. Sobernheim and Murata (1924) estimated the minimal lethal dose of a culture of *B. anthracis* when administered by different routes. They found that about 1/10,000 of a loopful would produce death when introduced intraperitoneally or intravenously, about 1/100,000 of a loopful when introduced subcutaneously or intracutaneously and about 1/1,000,000 of a loopful when injected intramuscularly.

It is obviously difficult to avoid infecting the skin when making injections into any subjacent tissue, and this objection has been urged against those who have recorded successful infection by some route other than the intracutaneous; but Besredka's assumption can hardly be reconciled with the observation that a *smaller* dose of bacilli is required to produce a fatal infection when the inoculation is made into the muscles than when it is made directly into the skin. Similarly, Basset (1925) was unable to demonstrate any superiority of the intracutaneous as compared with the subcutaneous or intramuscular route. Muller (1925) has recorded results which are directly opposed to those obtained by Plotz. He states that, several years before his paper was published, he had inoculated a pigeon's egg with anthrax bacilli, sealed the opening, and introduced the egg into the peritoneal cavity of a rabbit. Five weeks later this animal died from anthrax. Unable to reconcile this result with Besredka's views he carried out further experiments of the same type, but with a slightly different technique. Finding that anthrax bacilli would grow slowly through the pores of an L_1 candle, he placed cultures of the organism in such candles, sealed them carefully, and introduced them into the peritoneal cavities of nine rabbits. Seven of these animals died after intervals varying from 6 to 51 days. This long incubation period would appear to preclude an infection of the skin occurring at the time of the introduction of the candles. Burke and Barnes (1931) have utilized the bactericidal action of gentian violet in an attempt to produce an initial subcutaneous infection with *B. anthracis* unassociated with any primary infection of the skin. A guinea-pig was injected with a young culture of *B. anthracis* by introducing the needle through the skin of the abdominal wall in one flank, and then passing it subcutaneously across the abdomen to the other flank, where the inoculum was deposited. The point of the needle was then withdrawn to just beneath the skin puncture, the syringe barrel was detached, another barrel containing a 1 per cent. solution of gentian violet was attached, and the dye was introduced into the puncture as the needle was withdrawn. The guinea-pig died in 56 to 72 hours. The characteristic gelatinous infiltration occurred at the point where the inoculum had been deposited, not at the point where the needle had been inserted. Cultures from the dye-stained area about the site of the skin puncture remained sterile. It seems fair to conclude that Besredka's contention with regard to the unique susceptibility of the skin to anthrax has not been proved. Besredka and his colleagues (Besredka 1924*a*, *b*, Besredka and Urbain 1924) have also attempted to immunize the skin against other bacteria. They applied filtrates from broth cultures of staphylococci or streptococci to

the shaved skin of rabbits, and tested the resistance of these areas of skin to subsequent infection with the homologous organisms. From their results they conclude that this procedure confers a high degree of local immunity. In later reports they, and others, have recorded valuable curative properties in these filtered cultures—the so-called "anti-virus" preparations—but these observations do not bear directly on the problem at issue, and need not be further considered.

We need not doubt that various procedures of this kind may raise the resistance of local areas of skin to subsequent infection; but it by no means follows that this is associated with an increase in the resistance of the body as a whole.

Thus, Hach, Borodaj and Melnyk (1928) state that staphylococci, after intravenous inoculation into rabbits, tend first (3–3½ hours) to accumulate in the spleen, few remaining in the blood and few being present in the skin. As the infection progresses towards its rapidly fatal issue the distribution changes; the number of cocci in the spleen rapidly diminish, the number present in the skin show an enormous increase. Multiple intradermal inoculations of a staphylococcal filtrate, preceding the intravenous injection, prevented this rapid accumulation of staphylococci in the skin (Hach and Melnyk 1928); but there was no effective immunity, the rabbits dying of typical acute staphylococcal infection.

Is local immunity specific or non-specific?

We may now return to the more general question of the nature of the mechanisms that determine those types of local immunity that have been shown to exist, and inquire particularly whether they are in the main specific or non-specific.

Cobbett and Melsome (1898) found that a local non-specific inflammatory reaction, such as that following the injection of mustard oil, would effectively protect the skin of a rabbit's ear against subsequent attempts to produce experimental erysipelas.

Amoss and Bliss (1927) noted that the localized resistance following an erysipelatous lesion was not sharply specific. It was effective against strains of hæmolytic streptococci antigenically dissimilar from the strain producing the initial lesion, and to a less extent against *Staphylococcus aureus*. Rivers and Tillett (1925), studying the properties of the serum derived from the rabbits in which they had produced a localized antistreptococcal immunity, found that such immune serum injected into the skin 24 to 48 hours before the injection of living streptococci into the same site rendered this site relatively refractory to infection. Similar results were, however, obtained by the injection of normal serum, or of ordinary nutrient broth, though the protection in this case was less effective and quite non-specific. Mallory and Marble (1925) prepared filtrates from cultures of staphylococci and streptococci. With these they infiltrated localized areas in the skin of a rabbit, and found that such treatment conferred a strictly localized but non-specific immunity lasting for 5–6 weeks. This increased resistance was, however, little if any greater than that conferred by the infiltration of the skin with sterile broth. Analogous results have been recorded by Miller (1927), Freedlander and Toomey (1928), Hanger (1930), Toomey and Freedlander (1931), and Bridré and Bardach (1936).

Freedlander and Toomey carried out a detailed histological study of the reactions induced in the cutaneous tissues by the application of compresses of sterile broth, and of the reactions that followed the subsequent injection of *Staphylococcus aureus* into these treated areas. They found that the application of the broth compresses to the abdominal wall of the guinea-pig, for a period of 48 hours, produced a localized œdema, a marked proliferation of histiocytes and a moderate exudation of polymorphonuclear and small mononuclear cells. The cellular response to the subsequent injection of *Staphylococcus aureus* differed widely in treated and untreated animals. In the broth-prepared animals there was an increase in the histiocytes and fibroblasts with a dense exudation of poly-

morphonuclear cells, which showed little tendency to degenerate. The histiocytes phagocytosed the staphylococci at an early stage of the reaction, while the proliferating fibroblasts tended to wall off the lesion. In the control animals the œdema following the bacterial infection was more marked, there was little proliferation of histiocytes or fibroblasts, and the polymorphonuclear cells, which collected in considerable numbers during the earlier stages of the reaction, showed a marked tendency to undergo degeneration at a later period. The reaction was in general more severe and destructive and far less localized.

It would seem justifiable to conclude that there is an important non-specific element in the local immunity that follows the injection into the skin of various reagents, or its treatment with compresses, etc., and that this non-specific increase in resistance is determined in large part by the mobilization of various phagocytic cells, and perhaps by changes in the local capillaries and lymphatics.

Non-specific immunity of this type is by no means limited to the skin.

Pfeiffer and Issaeff (1894) showed that the injection of such materials as broth, peptone solutions or urine into the peritoneum of the guinea-pig would so alter the local conditions that the animal became resistant to the intraperitoneal injection of living cholera vibrios in doses fatal to untreated control animals. It is not justifiable to regard this particular non-specific immunizing reaction as local, in the same sense as the cutaneous reactions considered above, since material injected into the peritoneum is rapidly transferred to the blood stream, and a generalized non-specific response of the type discussed in Chapter 52 may well be concerned, in addition to any possible local effect.

Gay and Morrison (1923) injected various substances intraperitoneally into rabbits, and injected living streptococci 24 hours later. They found that certain substances, such as meat-extract broth, afforded marked protection, while others, such as aleuronat, did not. They noted that the substances which conferred protection were those that produced exudates in which macrophages were particularly numerous, while those that produced exudates containing numerous polymorphonuclear cells but few macrophages were far less effective.

Oerskov and Kauffmann (1936) and Oerskov (1940) record examples in mice of an intraperitoneal immunity to Vi forms of *Salm. typhi* and to *Sh. shigæ* that could be induced non-specifically by the injection of living or dead preparations of bacteria having no antigenic relationship to the infecting strain. Philipson (1937) has made an extensive study of the immunity to *Sh. shigæ* induced in mice by vaccines of *Salm. paratyphi B*. He found that a relatively high degree of non-specific immunity to intraperitoneal *Sh. shigæ* was demonstrable 8 hours after an intraperitoneal dose of vaccine, and lasted 3–9 days. When two doses of vaccine were given at a 9-days' interval, the immunity was demonstrable 2 hours after the second dose, and lasted up to 21 days. Thus the period of induction of the immunity was shortened, and its duration lengthened, by the re-vaccination. An intraperitoneal immunity of this degree, demonstrable 2 hours after the second dose of vaccine, could not be induced by intravenous vaccination and re-vaccination, nor by intravenous vaccination and intraperitoneal re-vaccination. Some immunity, however, followed intraperitoneal vaccination and intravenous re-vaccination, indicating that the stimulating effect of re-vaccination could be elicited in the peritoneum by blood-borne vaccine. Though the immunity was clearly non-specific, the stimulating effect of re-vaccination was in some way dependent on specific factors, for vaccination by one species of bacterium, and re-vaccination by an antigenically unrelated bacterium, was not followed by any significant increase in immunity.

Walsh and Cannon (1936) immunized rabbits against intranasal infection of the lungs with Type I pneumococci, by five or more daily instillations of formol-killed Type I pneumococci. The immunity was specific, though it appeared before antibodies were demonstrable in the blood of the animals. Cannon and Walsh (1937) recorded experiments which point to the induction of a local, perhaps specific, immunity in the lung by

this procedure—an immunity apparently dependent on an increased resistance to the passage of the cocci from the alveoli into the tissues (see also Exchaquet 1936). Similar results were obtained by North and Anderson (1942) who found that intranasal *H. pertussis* vaccines were more effective than intraperitoneal vaccines for immunizing against intranasal *H. pertussis*. They concluded, however, that the superiority of the intranasal route was due to non-specific immunity, since the antibody response to vaccination by either route was similar. Intranasal vaccines made of organisms more or less related to *H. pertussis*—*H. influenzæ*, *H. parapertussis* and *H. bronchisepticus*—were also effective. No immunity resulted from instillation of staphylococci or pneumococci, but a *Bact. coli* vaccine was highly effective. The efficacy of the *Bact. coli* appeared to depend on its capacity to induce a histological reaction in the lung similar to that induced by *H. pertussis*. Serologically speaking, the effect was non-specific. It is salutary to reflect that, had the test with *Bact. coli* been omitted, the evidence would have been of the kind we are accustomed to regard as a reasonable indication of the specificity of a particular immunizing effect.

It should be noted that the local changes associated with inflammation are not always protective. They may even increase susceptibility. Thus Olitsky and Schlesinger (1941) enhanced the infectivity of herpes virus for mice by first inducing œdema at the inoculation site with hypertonic salt solutions. Full inflammation induced by turpentine, on the other hand, was protective.

It is clear, then, that there is an element, in some cases a strong element, of non-specificity in local immunity. Its existence by no means excludes the possibility that specific factors are also operative. On this point the evidence is scanty, though, as we have seen in Chapter 50, there are indications that specific antibody formation occurs, not only in local lymph nodes, but in the skin also. The few experiments recorded on local antibody formation in the skin suggest that a conspicuous local proliferation of cells is a necessary preliminary to the formation of detectable antibody. It is possible, however, that an immunologically effective local response might take place in the absence of a gross proliferative lesion, and contribute to a specific local immunity.

Is local immunity necessarily associated with a cellular or tissue immunity in the strict sense?

There is, as has been pointed out above, no necessary correlation between local immunity and cellular immunity in the sense here implied—an immunity which belongs to the cell as such apart from its immediate environment and, in particular, apart from any antibodies which may be present in the tissue fluids in its immediate neighbourhood. A cellular immunity might well be widespread in its distribution, and effects. A local immunity may be due to a variety of factors other than an acquired resistance of particular cells.

None of the observations outlined above necessitates the assumption that a cellular immunity is involved; but all of them are quite compatible with its existence.

In this relation we may recall certain facts in regard to the formation and distribution of antibodies, and the phenomenon of anaphylaxis and hypersensitiveness. The serum antibodies, wherever they are produced, are rapidly distributed throughout the tissues, and are in part fixed by various tissue cells. We know almost nothing of the mechanism of this fixation, or of how the antibodies are distributed among the tissue cells. We do know, however, that a cell that has taken up its quota of a given antibody reacts specifically to the presence of the

corresponding antigen; and this mechanism would seem to be involved in those allergic reactions that are associated with infection and immunity. A "cellular immunity" of this type, and conditioned in this way, almost certainly occurs; though we have, at the moment, no knowledge of what part it plays in the mechanisms of immunity in general.

SUMMARY

The evidence available appears to justify the following conclusions.

(1) By the injection of various materials into the tissues, and by certain analogous procedures, it is possible to induce an immunity which is confined to the neighbourhood of the treated area, and is not shared by the body as a whole.

(2) This immunity appears to be distributed in accordance with the area involved in the original inflammatory reaction, not in accordance with the distribution throughout the affected part or throughout the body generally of one particular type of cell. There is good evidence of a localized immunity affecting an *area* of skin, or a particular serous sac. There is no adequate evidence of an immunity affecting the skin as a whole, or the intestinal mucosa as a whole, or serous surfaces as a whole, except as the expression of a general immunity.

(3) It follows that we cannot accept the conclusion that a localized immunity of the latter type is an essential factor in a general immunity. Even had an induced skin immunity, or mucous membrane immunity, been shown to exist, our knowledge of the general defence mechanisms of the body would prevent us from regarding such a local increase in resistance as the essential factor in antibacterial immunity.

(4) All the evidence suggests that non-specific factors are of primary importance in those instances of local immunity that have been submitted to experimental study. It would appear to be the initial inflammatory reaction as such, and the cellular changes which persist for some time after it has subsided, that determine the relative resistance of the treated area to subsequent experimental infections. In particular it would seem that any treatment which induces a local mobilization and concentration of histiocytes will confer on the treated area an increased resistance, which will last as long as the local cellular changes persist. There is some evidence that with the decline of the cellular change, the treated area remains for some time more responsive than normal to non-specific immunization.

(5) When a localized area is repeatedly treated with a particular bacterium, a general immunity is often superimposed on the local immunity. There is nothing in the evidence at present available to suggest that such general immunity, in so far as it is specific, differs essentially from the specific antibacterial immunity that we have considered in earlier chapters.

(6) The problem of the existence of a cellular, as opposed to a humoral, immunity is distinct from that of the existence of a local, as opposed to a general, immunity. There is nothing that compels us to assume the existence of a cellular immunity; but it would accord well with several experimental findings. If such a mechanism plays an essential part in immunity, it seems quite possible that it is conditioned by the fixation by various tissue cells of specific antibodies, and is hence only a humoral immunity at one remove.

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CHAPTER 54

THE INFLUENCE OF DIET, FATIGUE, CHANGES IN TEMPERATURE AND HUMIDITY, CHEMICAL AND CHEMOTHERAPEUTIC AGENTS AND OTHER FACTORS ON GENERAL OR LOCAL IMMUNITY

The Influence of Diet on Immunity

It will be convenient to discuss first the effects produced by deficiency or excess of the accessory food factors, or *vitamins*.

Many of the reports of the action of vitamins on the immune reactions in man and animals are contradictory, especially those of earlier workers in the field. Doubtless some of the contradictions are due to lack of knowledge of what constitutes a deficiency or an excess of a particular dietary factor, and to variations in the conditions of the experiments; but one of the most likely sources of error is the complexity of the vitamin preparations used. It is perhaps possible to say with conviction that a certain vitamin is in excess or deficient in a given diet, but the presence or absence of active amounts of other essential food substances is often problematical. In a few cases, as with Vitamin C, the difficulty may be overcome by comparing the effect of the synthetic vitamin with that of a preparation from natural sources; for though neither can with certainty be declared free of other vitamins, it is unlikely that the same contaminants will be present in both. In other cases we must rely on the chemical and biological evidence that the vitamin under test is a single substance free from contamination. The interpretation of vitamin work is often complicated by ignorance of the nutritional state of the animals under test. There may be, for example, a large difference between the optimal vitamin content of the tissues, and the level at which signs of vitamin deficiency appear; and of two apparently normal animals, one may have ample reserves of a vitamin, while the other will become clearly abnormal after only a short period of dietary deficiency. In these circumstances, discrepant results of experimental variations in vitamin intake are to be expected unless there is careful control of all the significant nutritional variables.

Another source of error has been demonstrated, particularly with regard to the vitamins of the B complex: namely, the contribution of the microbial flora of the gut to the nutrition of the animal under test. The synthesis and destruction of vitamins by intestinal bacteria is discussed in Chapter 90. We may note here that the intestinal flora may vary widely in its vitamin production, and that the bacterial vitamins may supplement, or even replace, the dietary supply of an essential vitamin. We need not labour the complications introduced into nutritional experiments by the normal bacterial flora of the intestine. To take one instance, it will be evident that the addition or omission of a non-vitamin dietary con-

stituent, by inducing a gross variation in the intestinal flora, may profoundly alter a vitamin balance that is largely maintained by microbial activity in the intestinal canal; and the consequences of the alteration may be attributed directly to the change in diet.

Variations in the Intake of Vitamin A.—In 1909 Knapp described a curious condition of the eyes developing in rats fed on a deficient diet. This condition (xerophthalmia) is characterized by a thickening and drying of the superficial epithelial covering of the eye, patchy in distribution, and associated in its later stages with a subacute bacterial infection of the underlying tissues. Knapp did not ascribe these lesions to any specific dietary deficiency; but we know now that the essential factor in its causation is lack of the fat-soluble Vitamin A, and Knapp's protocols show that the diet he employed was, in fact, deficient in this constituent. Similar lesions have since been described by many other observers, not only in the rat but in other animals, such as the rabbit (Nelson and Lamb 1920, Boock and Trevan 1922) and the dog (Steenbock, Nelson and Hart 1921). Particularly interesting is the account of Bloch (1921, 1928) of the occurrence of numerous cases of xerophthalmia in Danish children during the 1914–18 war, when the supply of animal fats to the general population, and in particular to the dwellers in large towns, was greatly restricted. The increased susceptibility to bacterial infection that results from Vitamin A deficiency is, however, by no means limited to the superficial tissues of the eye. In describing the condition of the children in Copenhagen Bloch remarks: "It is absolutely characteristic of these dystrophic children how little they are able to withstand infections, and how quickly they die of serious intercurrent fever." The most important killing disease that developed among them was broncho-pneumonia. Blegvad (1923) records the following incidence of serious complicating infections among the cases of xerophthalmia—broncho-pneumonia 63, bronchitis 45, pyuria 42, otitis media 30. These clinical observations are in entire accord with the results obtained in dietary experiments on animals.

The liability of rats fed on a diet deficient in Vitamin A to develop intercurrent infections, such as glandular abscesses, suppurative arthritis, suppurative otitis, pyelonephritis and broncho-pneumonia, has been noted by many observers (Drummond 1919, Hess *et al.* 1921, Cramer 1923, 1924, 1927, Gross 1924, Wolbach and Howe 1925, Green and Mellanby 1928, 1930, Gudjónsson 1930). The more precise knowledge that has accrued within recent years in regard to the chemical nature of certain of the vitamins has made it possible to demonstrate clearly that the fat-soluble vitamin concerned is A, not D (see Goldblatt and Benischek 1927, Green and Mellanby 1928); and to establish, in this respect as in others, the functional similarity of Vitamin A and carotene (Green and Mellanby 1930).

These observations on the increased frequency of spontaneous infections have been confirmed and extended by testing the resistance of animals kept on an A-deficient diet to experimental infection (Werkman 1923*b*, Hotta 1928, Reiter 1929, Lassen 1930, 1931, 1932, McClung and Winters 1932, Greene 1933, Robertson 1934). Lassen's studies are of particular interest, since the course of infection in the avitaminotic and control animals was followed in considerable detail. Rats were reared on a diet lacking Vitamin A, and were then infected, together with control rats of the same age fed on a complete diet, by administering living cultures of *Salm. typhi-murium* by the mouth, or by subcutaneous or intravenous injections. The avitaminotic rats showed a significantly lower resistance than the normal

controls. In the experiments in which the bacilli were administered by the mouth, the course of the infection was studied by the technique employed by Ørskov, Jensen and Kobayashi (see p. 1046). The rats were killed at various intervals after infection and cultures taken from the intestines, Peyer's patches, mesenteric glands, liver, spleen, lungs and heart's blood. In the normal rats the infection tended to be limited to the Peyer's patches and mesenteric glands, with some extension to the liver and spleen. In the avitaminotic rats it tended to become generalized, and to produce a fatal bacteræmic infection. In the few experiments in which the bacilli were injected intravenously, the avitaminotic rats showed the same initial clearing of the blood stream as did the normal controls, but in the former this initial clearance was followed by a secondary bacteræmia and a fatal generalization of infection.

By adding irradiated ergosterol to the Vitamin A-deficient diet and noting that this procedure did not prevent the fall in resistance to experimental infection, Lassen (1931) was able to show—as Green and Mellanby had done in the case of spontaneous infections—that the effect of removing all fat-soluble vitamins from the diet was due to the absence of Vitamin A.

It may be noted, and this is a point of some importance, that Lassen found it necessary to induce a pronounced degree of avitaminosis before he could demonstrate a well-marked break in resistance. He states that a slight decrease in resistance occurs after 2 to 3½ weeks on a Vitamin A-free diet, at a time when the animal shows no clinical evidence of avitaminosis, but that the clear and significant fall in resistance sets in suddenly at a later stage, coincidentally with the appearance of xerophthalmia.

The mechanism that underlies the decreased resistance of animals deprived of Vitamin A is as yet far from clear. Cramer (1923, 1924) has described atrophic changes in the intestinal mucosa of avitaminotic rats, and believes that the lesions facilitate the passage of bacteria from the intestines to the tissues. Wolbach and Howe (1925) record striking changes in the surface epithelium in certain situations, though they locate these lesions in the lining membranes of the nares, trachea, bronchi and genito-urinary tract, rather than in the intestine. These observations would suggest that the increased susceptibility to infection associated with A-avitaminosis may be due, in part at least, to a failure of the first-line defence mechanisms discussed in Chapter 45; but such a view is difficult to reconcile with other evidence on record. Thus Lassen (1931) found no difference in the rate at which bacteria passed from the intestine to the mesenteric glands *via* the Peyer's patches and lymph follicles, as between normal and avitaminotic rats. It was in the subsequent course of events—the fatal generalization of infection as opposed to its successful localization—that the avitaminotic animals displayed their lack of effective resistance. Moreover (Hotta 1928, Reiter 1929, Lassen 1930, 1931, 1932) such animals are more susceptible than normal controls to the subcutaneous, intraperitoneal and intravenous injection of bacteria—in which the first line of defence is artificially ruptured—as well as to the administration of pathogenic bacteria by the mouth (see also Stryker and Janota 1941). On the other hand, Sternberg and Pillsburg (1937) were unable to detect any lowering of the resistance of rats to intradermal infections with staphylococci and streptococci, as the result of Vitamin A deficiency.

It would seem, therefore, that there must be some defect in the defence mechanisms of the tissues—in the efficient functioning of the reticulo-endothelial system itself, or in the conditioning or accessory action of the humoral antibodies—but of the nature of that defect we have as yet no knowledge. It may be noted in this connection that most workers have failed to find any satisfactory evidence that deprivation of Vitamin A—or indeed of any other vitamin—reduces the efficiency of antibody production in response to a

specific stimulus (see Zilva 1919, Werkman 1923*a*, *b*, Werkman, Nelson and Fulmer 1924, Cramer and Kingsbury 1924, Simola and Brunius 1933), though Greene (1933) records an apparently significant decrease in the ability of A-avitaminotic rabbits to produce hæmolysins for sheep's red cells and Jusatz (1936*a*) a depression in rabbits both of the bactericidal power of the blood and of the formation of precipitins for horse serum after prolonged feeding on a diet free from vitamins A and D. Lassen (1931, 1932) notes that the sharp difference in resistance between normal and avitaminotic rats cannot be demonstrated when both groups have been previously immunized by the injection of a heat-killed vaccine, which indicates that the antibody-producing mechanism is still functioning with effect. Boynton and Bradford (1931) tested the resistance of rats to artificial infection with a bacillus of the Friedländer group, and found that it was lowered by Vitamin A deficiency even before signs of avitaminosis had developed.

With progressive deficiency Gellhorn and Dunn (1937) observed a progressive fall in the phagocytic index of rat-blood, which was reversed when Vitamin A was restored to the diet. The association of this effect with deficiency is dubious, since the deficient rats suffered from spontaneously acquired infection, which may itself have affected the phagocytic index. The significance of lysozyme in defence against infection is not clear (see Chapter 45) but it may be noted that in deficient rabbits Sullivan and Manville (1937) found a retention of lysozyme in the intestinal tissues, and a diminution in the mucous secretions.

Observations have been recorded suggesting that the deprivation of Vitamin A renders animals more susceptible to the injection of bacterial toxins, as well as to the invasive action of living bacterial cells (Werkman, Baldwin and Nelson 1924, Schubert 1928). Torrance (1936), however, in a careful study of the Vitamin A content of the liver in guinea-pigs, found no association between vitamin level and susceptibility to diphtheria or tetanus toxin (see also Chalier and Jeune 1938).

It is possible that the whole effect of Vitamin A deficiency is indirect. Kligler, Guggenheim and Henig (1945) found, in accordance with a number of other observers, that resistance of rats and mice to infection—in their case with *Salm. typhi-murium*—was not significantly lowered by a partial Vitamin A deficiency, but only by a complete avitaminosis. Avitaminosis was, however, invariably associated with declining weight in the experimental animals. In other words, the decline in resistance appeared to be due to starvation, a conjecture supported by the fact that rats whose food intake was restricted to the same amount as that eaten by avitaminotic rats, while the Vitamin A intake was normal, were as susceptible as the avitaminotic animals to the salmonella infection.

We have been concerned so far with a comparison between animals fed on a normal diet and those deprived of Vitamin A. The former have clearly an advantage; but it is by no means a necessary corollary that an excess of Vitamin A will raise the resistance above normal level.

There are as yet no adequate experimental or field data on which a sound opinion on this further problem can be based. It is intrinsically a difficult one, since it really resolves itself into comparisons of resistance over a wide range of different levels of Vitamin A intake. The amount that is required for normal growth may fall short—even if not far short—of the amount required for optimal resistance.

Among other experiments that have a bearing on this point are those of Webster and Pritchett (1924) on the influence of diet on susceptibility to experimental infection with *Salm. typhi-murium*. They compared the resistance of mice fed on the ordinary diet of the Rockefeller Institute—a daily ration of bread, soaked in fresh, pasteurized, Grade B milk, supplemented by two weekly feedings of an oatmeal and buckwheat mixture and one weekly feeding of dry biscuit—with that of mice fed on a modified McCollum diet containing wheat flour, casein, milk powder, butter fat and salts. Mice kept on these diets were tested

in groups of 10–36 animals, by the injection through a stomach tube of 2–5 million *Salm. typhi-murium*. The results indicated that the mice fed on the McCollum diet were significantly more resistant than those fed on the ordinary laboratory diet. Pritchett (1927) records a more numerous series of experiments along similar lines, in which an attempt was made to assess the influence of the different constituents of the McCollum diet. The increased resistance of the mice fed on this diet was in general confirmed; but the effects produced by varying its constitution were not easy to interpret. For instance, the entire withdrawal of butter fat—the only source of Vitamin A—left the mice more resistant than the mice on the ordinary laboratory diet, though less resistant than those on the full McCollum diet. On the other hand the addition of butter fat to the normal laboratory diet was followed, in four of seven trials, by a lowering of mortality as compared with the control group; and a similar result was obtained by the addition of cod-liver oil. In a later series of experiments on the effect of diet on the epidemic spread of mouse typhoid Webster (1930) records results from which he concludes that the substitution of the McCollum diet for the ordinary laboratory diet increases the resistance of mice to contact infection.

In another series of observations along similar lines (Topley, Greenwood and Wilson 1931) different results were obtained. Mice fed on a basal diet of whole-wheat flour, casein, butter and a salt mixture showed no advantage over mice fed on a diet of whole oats, milk and water as regards their ability to resist infection during an experimental epidemic of *Salm. typhi-murium* infection. The addition of an excess of fat, butter or lard, or of a Vitamin-A concentrate to this diet appeared to react unfavourably on the mice at risk. The unfavourable action of these artificial diets was not manifest when the mice were infected by intraperitoneal inoculation. Under these conditions there was a suggestion that the mice fed on the usual laboratory diet of oats, milk and water were slightly less resistant than the other groups, but the differences observed were quite insignificant. In order to avoid the possible complication introduced by the excess of fat in the vitamin-rich diets, other experimental epidemics were initiated in which the vitamin content of the normal laboratory diet was increased by the addition of a ration of carrot or of cabbage. The mice receiving carrots appeared to be at a disadvantage as compared with those on the normal diet; the addition of cabbage produced no significant change in mortality. It may be noted that Lassen (1931) records that a considerable excess of shark-liver oil in the diet caused a slight decrease in the resistance of rats to experimental paratyphoid infection.

These experiments are considered here because some of them at least have a bearing on the effect of an excess of Vitamin A over the amount required to maintain growth and apparent well-being; but it would in truth be unwise to lay much stress on their significance, since it is clear that the diets employed varied in many ways besides their differences in Vitamin A content. It does, however, seem fair to conclude that no adequate experimental evidence has yet been produced that an excess of Vitamin A over the level required to prevent all overt signs of avitaminosis induces an increased resistance to bacterial infection.

Little evidence is available with regard to the effect of increasing the amount of Vitamin A supplied to human subjects. The collection of such evidence clearly demands special conditions. The infections associated with child-birth afford opportunities for such a trial, and are of particular interest because of the obvious possibility that pregnancy may make a large call on the vitamin reserves. Mellanby, Green and their colleagues (Green *et al.* 1931) record a trial in which 275 women received a Vitamin-A concentrate by mouth during the last month of pregnancy, the incidence of varying degrees of morbidity among this group during the puerperium being compared with the corresponding figures for a control group of 275 women not so treated. In every comparison the treated group fared better than the untreated. Thus 19·2 per cent. of the treated women suffered from pyrexia,

as against 30·9 per cent. of the untreated; while for severe pyrexia the figures were 1·5 per cent. and 3·6 per cent. respectively. The evidence so far as it goes clearly suggests that the administration of Vitamin A in large amounts has some prophylactic value against the risk of puerperal infection.

There is no obvious reason for supposing that the administration of excess of Vitamin A would exert a beneficial effect on an already established infection; and most of the scanty evidence available is in accord with the *a priori* probabilities. Mellanby and Green (1929) reported a few cases from which they concluded that a therapeutic effect was exerted in puerperal fever; but the studies of Thomas (1931) on a far larger series of puerperal cases showed no benefit, either in mild or serious infections. Orenstein (1932) failed to find any evidence that the administration of a highly potent preparation of Vitamin A has a beneficial effect on cases of pneumonia occurring among the native workers on the Rand mines. Over a 1-year period alternate cases were treated with this preparation. Of 375 treated cases 10·13 per cent. died; of 389 untreated cases 10·28 per cent. died.

Variations in the Intake of Vitamin B.—The Vitamin B complex includes defined vitamins like Vitamin B_1, or thiamin, which is relatively heat-labile, and the heat-stable B_2 complex, which includes nicotinic acid, riboflavin, pyridoxin, pantothenic acid, biotin, and other substances. It would appear both from clinical records and from experimental studies that a deficiency in the intake of Vitamin B_1—the antineuritic vitamin—has no effect on resistance to infection at all comparable to that produced by a lack of Vitamin A. In the earlier records there are suggestions that B-avitaminosis is associated with a minor increase in susceptibility to infection, but the evidence is of very doubtful significance (see Werkman 1923*a*, *b*, Werkman, Baldwin and Nelson 1924, Lassen 1929, 1931, 1932, Robertson 1934).

The effect of a prolonged suboptimal intake of the vitamin B complex was studied in rats by Drummond and others (1938). The deficient rats had feebler reproductive powers, were shorter-lived, and post mortem showed a significantly higher incidence of gastro-intestinal lesions, than those in a control group on a full diet. Nevertheless, they suffered from no epidemics, and the incidence of bronchiectasis, the only infective disease to which the rats were prone, and of sporadic pneumonia and abscesses, was the same in deficient and control groups. Rose and Rose (1936) compared the course of artificial *Staph. aureus* bacteræmia in 8 normal and 8 B_1-deficient dogs. The deficient dogs lost weight more rapidly, and suffered a more prolonged bacteræmia, but there was no significant difference in the recovery rates in the two groups. They found no depression of resistance to *Cl. welchii* toxin in deficient dogs (Rose, Rose and Kolmer 1936). Janota and Dack (1939) record that the anæmia, leucopænia, gingivitis, œdema and diarrhœa which follow B_2 deficiency in monkeys, is associated with the spontaneous appearance of *Sh. flexneri* in the stools. Rats fed on B-deficient (Lamb 1935) or thiamin-deficient diets (Badger, Masunanga and Wolf 1940) exhibit a decreased resistance to rat leprosy. Woolley and Sebrell (1942) maintained mice on thiamin- and riboflavin-deficient diets for 2–3 weeks, and found a diminished resistance to intranasal infection with Type I pneumococci. Robinson and Siegel (1944), using comparatively small numbers of animals, studied the effect of restricted B vitamin intake on rats injected intratracheally with a mixture of mucin and Type I pneumococci. Compared with rats on a defined, "synthetic" diet containing adequate amounts of known B vitamins, neither riboflavin- nor pantothenic acid-deficient rats were less resistant; thiamin deficiency, and to a less significant degree, pyridoxin deficiency, lowered the resistance. Rats on a "normal" stock diet, it should be noted, were as susceptible as thiamin-deficient rats.

Among virus diseases the effect of deficiency varies. Cowdry, Lucas and Neff (1935) found no diminution of resistance to injection with herpes virus in B_1- and B_2-deficient

rats. Kuczynski (1937) increased the survival rate of normal mice injected with yellow fever virus from about 20 per cent. to about 75 per cent. by increasing the dietary thiamin. It is doubtful whether these effects are specific. For example, Sabin (1941) has shown that though thiamin or B-complex deficiency delays the normal acquisition by mice of an adult resistance to encephalitis induced by the virus of vesicular stomatitis, a similar delay occurs when food is restricted, and the dietary B vitamins are adequate. In this connection we may note that Cottingham and Mills (1943) found a lowered phagocytic activity in the blood of rats which were deficient in thiamin, riboflavin, pyridoxin, pantothenic acid or Vitamin C only when the deficiency in each case was sufficient to retard the growth of the animals.

Experimental poliomyelitis provides an example of a disease in which vitamin deficiency is apparently beneficial. Compared with normal mice, the incubation period, and the incidence of mortality and of paralysis among thiamin-deficient animals, are low (Rasmussen *et al.* 1943, 1944, Foster *et al.* 1944). Restriction of diet intake produces a similar but slightly less marked increase in resistance. Sprunt (1942) also records that undernourished rabbits are less susceptible than fully-nourished rabbits to vaccinia virus, and points out that viruses, which are highly adapted intracellular parasites, may be peculiar in requiring the full health of the host's susceptible cells for their proliferation. This peculiarity is not, however, confined to virus infections, for pantothenic acid deficiency is reported to be associated with an increased resistance to experimental infection with Type I pneumococci (West *et al.* 1944).

We cannot doubt that variations in the intake of B vitamins influence the course of infection by bacteria and viruses, though we cannot predict what the effect will be in any one type of infection; nor is it always possible to say the decrease in resistance is a specific vitamin effect. It should be noted that thiamin and riboflavin deficiency decreases resistance in rats to worm infection (Watt 1944) and biotin deficiency the resistance of birds to malaria (Trager 1943).

In man, riboflavin deficiency is associated with breakdown, fissuring and sometimes infection of the tissues at the angles of the mouth and in the naso-labial folds; and nicotinic acid deficiency (Sebrell and Butler 1939) with acute ulcerative stomatitis, in the lesions of which Vincent's organisms (see Chapter 80) may be found. A similar association of stomatitis, gingivitis and periodontal suppuration, with deficiencies of both the B_2 complex and Vitamin C, is reported in monkeys (Topping and Fraser 1939, Chapman and Harris 1941). There is little evidence to suggest that these infections are more than a secondary invasion of the superficial lesions associated with the vitamin deficiency.

Variations in the Intake of Vitamin C.—The evidence with regard to the effect of Vitamin C on resistance is peculiarly confusing and discrepant.

On the clinical side Hess (1920) stresses the frequency of infective complications in scurvy, while Bloch (1928) states that scorbutic children show no tendency to infection in any way comparable to that displayed by children suffering from xerophthalmia (see also Robertson 1934).

Fox and his colleagues (1940) found no difference in sickness rates of adult negroes receiving adequate Vitamin C and of those on a vitamin-deficient diet. Vogl (1937) reported that Vitamin C prevented the development of post-operative pneumonia, and that it hastened the convalescence of pneumonic patients (see also Sennewald 1938 Szirmai 1940). Beneficial results have been claimed also in the treatment of whooping cough (Ormerod and Unkauf 1937), though Gairdner (1938) in a carefully controlled test found no difference between untreated and vitamin-treated children. Bamberger and Zell (1936), Dieckhoff and Schüler (1938) and Szirmai (1940) also claim beneficial results in diphtheria. In most cases, however, the number of uncontrolled factors in these tests is large, and conclusions as to the specific action of the vitamin must be drawn with circumspection.

Glasebrook and Thomson (1942) provide one of the most clear-cut indications of a specific Vitamin C effect. They studied two groups of boys, one deficient in, the other saturated with, Vitamin C. They could find no difference in the incidence of common colds or tonsillitis in the two groups, but noted that deficient patients with tonsillitis stayed in hospital 16·7 days on the average, as against 16·0 days for the saturated patients. During the period of observation there were 17 cases of pneumonia and 16 cases of acute rheumatic fever among 1,100 deficient boys, and none among 335 saturated. The association of infection with low vitamin levels in animals or man is not in itself good evidence that the one is a direct result of the other, for in the infective state, especially when it is accompanied by fever, there is a decrease of the vitamin in the tissues; indeed, infection may precipitate scurvy in persons suffering from hypovitaminosis (see Abbasy *et al.* 1937, Harris *et al.* 1937, Fox 1943). The point is well illustrated in the attempts of Rinehart and his colleagues to reproduce the lesions of rheumatic fever in guinea-pigs. They found (1934) infection of scorbutic guinea-pigs with hæmolytic streptococci resulted in certain cardiac lesions which did not appear in scorbutic animals, or in normal animals infected with the streptococci. Moreover, in patients with rheumatic fever, the vitamin levels were unusually low (1936). McBroom and his colleagues (1937), on the other hand, observed the lesions in uninfected scorbutic guinea-pigs, provided that the scurvy was severe enough, and suggested that Rinehart's lesions were due to severe scurvy, precipitated in a non-specific manner by the superadded infection.

As regards spontaneous infections in guinea-pigs suffering from experimental scurvy, Hamburger and Goldschmidt (1922–23), Grant (1926, 1930) and Schmidt-Weyland and Költzsch (1927) state that they are very common. On the other hand, Holst and Frölich (1912) state that they are uncommon. The observations of Glenny and Allen (1921) on the occurrence of epidemic infection among a stock of guinea-pigs on a vitamin-deficient diet, and its suppression by the addition of a ration of green stuff, may perhaps be taken as relevant evidence, though we clearly cannot assume that C-avitaminosis was the determining factor.

As regards the resistance of scorbutic guinea-pigs to experimental infection, Findlay (1923), Werkman, Nelson and Fulmer (1924), Grant (1926) and Schmidt-Weyland and Költzsch (1927) record a slight increase in susceptibility. Findlay would ascribe this to the degenerative changes and feeble leucoblastic reaction seen in the bone-marrow of guinea-pigs suffering from chronic scurvy. Werkman, Nelson and Fulmer stress the association between lowered resistance and lowered body temperature.

The observations of Zinsser and Castaneda (1931) on the increased susceptibility of guinea-pigs and rats on a vitamin-deficient diet to experimental typhus infection may be referred to here. It is probable that the guinea-pigs were suffering from some degree of C-avitaminosis; but the fact that rats, which are generally regarded as being relatively insensitive to the deprivation of this vitamin, showed the same decrease in resistance makes it difficult to assess the significance of these results.

McCullough (1938) records an increased susceptibility to infection by *F. necrophorus* in deficient guinea-pigs, which only became evident when the scurvy was severe; the administration of Vitamin C was followed by prompt recovery. In man, major deficiency is associated with a retardation of fibroblastic activity and of wound healing (see Hunt 1941, Bourne 1942, 1944), and the possible association of vitamin deficiency and an inadequate cellular response to infection is evident in the observations of Meyer and Meyer (1944) on experimental *Staph. aureus* infection of guinea-pigs. In normal animals the abscesses produced were hard and circumscribed; those in scorbutic animals soft and with indefinite edges. The difference appeared to be due in the main to the extremely poor fibroblastic response in the scorbutic animals with a consequent failure in "sealing off" the abscess from healthy tissues.

Both bacteria and bacterial exotoxins are susceptible to the action of Vitamin C *in vitro*. The growth of diphtheria bacilli, streptococci, pneumococci, staphylococci, salmonellæ and *H. pertussis* is inhibited (von Gagyi 1936, Büsing 1939, Meyer 1939, Otani 1939).

Small amounts of diphtheria toxin are inactivated by Vitamin C (Harde and Philippe 1934, Greenwald and Harde 1935, Jungeblut and Zwemer 1935, King and Menten 1935, Widenbauer and Saretz 1936, Yokoyama 1938, Jungeblut 1941, Willison 1943). Jungeblut noted that the detoxication was increased in the presence of Cu ions. Petherick and Singer (1943) record a similar detoxication of *Cl. welchii* α-toxin, and of diphtheria and staphylococcal toxins, by Vitamin C in the presence of Fe, Cu, and Zn ions.

These observations have led to a number of tests on the protective effect of excess Vitamin C against diphtheria intoxication. The evidence for a protective action of the vitamin is conflicting. Neither Greenwald and Harde (1935), Widenbauer and Saretz (1936), Berger (1937) nor Zilva (1937) could detect any protective action in guinea-pigs; nor could Pakter and Schick (1938) detect any differences in the Schick-reactivity of normal children and children given massive doses of Vitamin C. Jungeblut and Zwemer (1935), King and Menten (1935), Lyman and King (1936) Yano (1938) and Jungeblut (1941), on the other hand, report that excess of the vitamin, given an hour or so before the toxin, will protect against 1–2 m.l.d. The protective effect was, however, inconstant. It appears that severe degrees of diphtheria intoxication reduce the vitamin content of the tissues (Torrance 1940; see also Lyman and King 1936). Vitamin C has little action on staphylococcal toxin (Mercier 1938) or that of *Cl. welchii* (Souto and Lima 1938), but inactivates the toxins of *Cl. tetani*, (Jungeblut 1937*c*, Kligler *et al.* 1938), *Cl. œdematiens*, *Cl. septicum* and *Cl. histolyticum* (Souto and Lima 1938). Souto and Lima claim that the vitamin has a prophylactic action in the animal against all four gas-gangrene toxins. Among the viruses we may note that Vitamin C inactivates herpes virus (Holden and Resnick 1936) and apparently increases the resistance of rabbits to dermal infection with the virus (Holden and Molloy 1937). It also inactivates vaccinia virus (Kligler and Bernkopf 1937). Jungeblut (1937*a*, *b*) has reported a marked diminution in the paralysis and death rates of poliomyelitic monkeys when treated with Vitamin C (see Chapter 87), but neither Toomey (1937) nor Sabin (1939) could confirm this result.

The *in vitro* action of vitamin C on infective agents and toxins can in most cases be ascribed to its considerable powers as a reducing agent. It seems unlikely that it could be given to the infected animal in sufficient quantities to produce in the tissues reducing intensities comparable with those in the test-tube, and we must ascribe any indubitable *in vivo* effect to a more complex action.

The same conflict of evidence holds with regard to the immunological consequences either of excess or deficiency of Vitamin C.

Lesné and Dreyfus (1911) and Zolog (1924) claimed that in guinea-pigs deficiency was associated with diminished sensitivity to anaphylactic shock. Cohen (1938) was unable to confirm these findings (see also Sartori 1925, v. Niekerk 1937). Several observers report a prophylactic effect of excess Vitamin C in guinea-pigs, both in the whole animal, and in isolated sensitized tissues (Lemke 1936, Solomonica 1936, Ungar, Parrot and Levillain 1937). Hochwald and Schwartz (1937), Raffel and Madison (1938) and Storck (1939) were unable to confirm these findings, and Dragstedt (Dragstedt *et al.* 1938) failed to find any effect of Vitamin C on hypersensitive dogs. Similarly with antibody formation; Jusatz (1936*b*) and Madison and Manwaring (1937) record enhancement of precipitin response in rabbits, and Raffel and Madison (1938) in guinea-pigs given excess Vitamin C. Hochwald and Schwartz (1937) found no enhancement in guinea-pigs immunized with horse serum, and Madison, Fish and Frick (1938) no effect on the response to bacterial antigens. (For a discussion of the relation between Vitamin C and serum complement, see Chapter 7.)

Variations in the Intake of Vitamin D.—The similar behaviour of Vitamin A and the anti-rachitic vitamin as regards their fat-solubility makes many of the earlier observations on the effect of a Vitamin D-deficient diet of doubtful significance.

Certain of the experimental studies that we have summarized above show that the addition of Vitamin D to a diet from which the fat-soluble vitamins have been removed does not prevent the lowering of resistance: indeed there is a suggestion in some of them (see Lassen 1931) that the addition of irradiated ergosterol increases the susceptibility of the animals receiving it. In other experiments Lassen found that rats on a rachitogenic diet not entirely lacking in Vitamin A showed no appreciable lowering of their resistance to experimental infection *per os*, even when the macroscopic and histological evidence of rickets was clear and unmistakable; and Freund (1932) was unable to demonstrate any significant difference in the resistance of rats and mice maintained on normal, Vitamin D-free or Vitamin D-excess diets. As we have noted above, a simultaneous deficiency of Vitamins A and D was found by Jusatz (1936*a*) to reduce the bactericidal power of the blood. Toomey (1937) protected monkeys against intestinally, but not intracerebrally, injected poliomyelitis virus by treatment with Vitamin D, but not with A and C, or the B complex. Sabin and his colleagues (1941), on the other hand, found that normal and rachitic monkeys were equally susceptible to the virus. By feeding puppies for 30–60 days on a diet containing excess of yeast and carotene, Frohring (1937) claimed to have increased their resistance to dog distemper so that none of them developed the disease when exposed in a distemper ward. The administration of a high vitamin diet apparently reduced the mortality in dogs that had contracted the disease. Scheunert and Petzold (1937) were unable to modify the susceptibility of puppies to experimental distemper by a diet rich in irradiated yeast, or to modify the severity of the disease by cod-liver oil or Vitamin B therapy. However, when the yeast diet was given to pregnant bitches, and maintained during weaning, the resistance of the puppies was increased.

If we attempt to assess the significance of the data at present available with regard to the influence of variations in vitamin intake on resistance to infection, we shall probably conclude (1) that there is good evidence that gross deficiency of Vitamin A is accompanied by a reduction of resistance; (2) that it is doubtful whether the addition of any extra source of Vitamin A to an ordinary unrestricted human or animal diet is likely to lead to any significant increase in resistance, save, possibly, in the special conditions of pregnancy, but that this question must await the answer of properly controlled trials carried out on an adequate scale; (3) that the evidence with regard to the effect of Vitamin C deficiency is so divergent that no sound conclusion is possible at the moment, though there are definite suggestions that animals such as the guinea-pig, that are peculiarly susceptible to the deprivation of this vitamin, are rendered more susceptible to infection when it is withheld; (4) that certain members of the B complex are associated with the factors that determine resistance and susceptibility; and (5) there is at present no satisfactory evidence that Vitamin D is associated with these factors.

The relation of resistance to infection and other members of the rapidly expanding class of accessory food factors has as yet hardly been explored in any precise manner, but the future will undoubtedly reveal significant relationships. (For more detailed general reviews of the literature see Lassen 1931, Clausen 1934, and Robertson 1934.)

The Influence of Other Dietary Factors on Resistance.

The variations in dietary factors which we review under this heading must not be understood to exclude variations in the vitamins. In many cases, the content of recognized vitamins in the diets is not itself known, and, in other cases, where they are known, effects that we wish to ascribe to certain energy-producing food materials may in fact be due to traces of unrecognized vitamins present in the diet. We may first summarize observations which seem to provide an unequi-

vocal experimental demonstration of an increase in resistance due to a change in diet, though the mechanism involved is, as yet, quite obscure.

Robertson and Doyle (1936) maintained three groups of rats on a basic diet of salts, cornstarch, vegetable fat, and vitamins in the form of cod-liver and wheat-germoils. Casein, wheat gluten and soya bean were added, respectively, to the diet in each group and the resistance of the animals was tested by intraperitoneal injection of *Salm. enteritidis*. The survival rates were 86 per cent. in the casein-fed animals, and between 30 and 40 per cent. in the two other groups.

Watson (1937) made a systematic study of the resistance of mice to infection with *Salm. typhi-murium*, and obtained significant results during the comparison of two diets, which differed in that 50 per cent. of the oatmeal supplied in one diet was, in the other, replaced by dried milk. In each case the diets were otherwise adequate and vitamins were supplied in the form of yeastrel and cod-liver oil. In repeated tests, carried out on statistically adequate groups of mice, it was found, with few exceptions, that the mice fed on the diet containing dried milk were significantly more resistant to the intraperitoneal injection of a toxic fraction isolated from *Salm. typhi-murium*. They were also more resistant to the administration by the mouth of living cultures of *Salm. typhi-murium*. They were not significantly more resistant to the intraperitoneal injection of living bacilli. The resistance to the injection of the toxin, and to the oral administration of moderately virulent cultures, was fully developed only in mice that had been fed since birth on the diet containing dried milk. It was detectable in mice that received the diet for three weeks before the test inoculation, but was in this case barely significant. The comparison was extended (Watson, Wilson and Topley 1938) to mice exposed to contact infection with *Salm. typhi-murium*. A significantly lower epidemic mortality occurred in the groups whose diets included the dried milk. The better diet apparently reduced the risk both of manifest, and of latent infection.

Church (1939) records results substantially in agreement with these using *Salm. enteritidis* as the infecting agent; he made the interesting observation that dietetic effects were far more readily produced in young mice if the mothers were also subjected to the diet from a few weeks prior to mating. Indeed, the only changes found to exert a significant effect when instituted first in the weaned mouse occurred as the result of a 75 per cent. decrease in the total mineral constituents or an 80 per cent. decrease in calcium content.

Reference to the protocols recorded by Webster and Pritchett (1924), by Pritchett (1927), and by Topley, Greenwood and Wilson (1931) will show that Watson's results were entirely compatible with the view that the milk powder in the diet was the component that determined the increased resistance of the mice receiving it, though this possibility was not in fact envisaged or discussed. How the effect is exerted it is not, as yet, possible to say. It seems, perhaps, more likely to be due to the effect on metabolism of a well-balanced diet, than to traces of any accessory substance in the dried milk; and it would appear that the tissue changes on which increased resistance depends take some time to develop.

Turning to studies that have so far yielded less definite and clear-cut results we may first consider certain surveys of a quite general kind, in which an attempt has been made to establish preliminary correlations that will serve to define problems rather than to solve them.

In this category fall the studies of Orr and Gilks (1931) on the health of two African tribes—the Masai and the Kikuyu—and related studies (Orr, MacLeod and Mackie 1931) on the effect of nutritional variations on the resistance of sheep. The Masai, particularly the males, live mainly on a diet of milk, meat and raw blood. The Kikuyu live almost entirely on cereals, roots and fruits. Apart from a marked difference in physique—the adult male Masai is 5 inches taller, 23 lb. heavier and 50 per cent. stronger than the Kikuyu

—there appears to be a significant difference in the frequency of certain types of infective disease; though the facilities for obtaining statistical data among native tribes are not of the kind that allow the collection of entirely convincing evidence. With this reservation, it would seem that pulmonary infections (bronchitis, pneumonia and phthisis) and tropical ulcer are far more frequent among the Kikuyu than among the Masai, while the latter are more prone to arthritis, presumably of an infective type. This difference in susceptibility to particular kinds of infection is associated with a low calcium content of the blood in the Kikuyu (9·4 mgm. per 100 ml. as against 11·4 mgm. among a small sample of Europeans in Kenya and 10–10·5 mgm. among Europeans in general). Samples of Masai blood could not be obtained on account of a tribal tabu. In the experiments on sheep, which were carried out in Scotland, animals were allowed to graze on different types of pasture, or fed on different diets in pens, and studies were made of the blood calcium, blood phosphorus, and (as an index of immunity *faute de mieux*) of certain of the natural agglutinating or lytic antibodies. The results, as one would expect, are by no means easy to interpret; but there appears to be a definite correlation between ample pasturage, a high calcium and low phosphorus content of the blood, and general well-being, and there is a suggestion that these conditions are associated with active lytic properties of the serum, while the agglutinins in certain experiments appear to have varied inversely as the lysins. In later experiments (Mackie *et al.* 1932) a slight indication was obtained that the animals on the poor pasture were more susceptible than controls to the intradermal injection of the toxin of the lamb dysentery bacillus.

It is noteworthy that experimental depletion of the serum proteins in dogs, by repeated bleedings and replacement of whole blood by erythrocytes alone, or experimental hypoproteinæmia and anæmia together, is associated with a high incidence of endocarditis, pneumonia and septicæmia (Robscheit-Robbins, Miller and Whipple 1943*b*). Mills and Cottingham (1943) found a marked decrease in the phagocytic activity of blood leucocytes in rats on a protein-deficient diet, and suggested that increased susceptibility to infection in these circumstances might be the result of depressed phagocytic powers.

The possibility that variations in the intake of *mineral salts* may have an influence on resistance has been explored by various experimental workers (Zilva 1919, Lange 1925, 1927*a*, *b*, Pritchett 1927, Hotta 1928, Kligler and Geiger 1928, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931), but, excepting the results of Church (1939) noted above, the evidence is highly confusing. We can, at the moment, do little more than suspend judgment, and note that a question has been asked, but not answered.

We may pass to the effect of a general deficiency of food, apart from qualitative changes. We are here in little better case, so far as experimental evidence is concerned. Hotta (1928) records a definite decrease in the resistance of mice kept on a starvation diet, while Lassen (1931) finds only a trivial decrease in resistance of rats suffering from a relative but well-marked inanition for a period of 17 weeks (see also Müller and Simons 1919, Bieling 1925, Bickert 1931).

Apart altogether from their effect on the defence mechanisms of the tissues, there can be no question that variations in diet alter the local conditions in the gastro-intestinal tract. We know (see Rettger and Cheplin 1921, Dudgeon 1926) that the ingestion of certain food-stuffs produces characteristic changes in the gastro-intestinal flora; and Arnold (1926, 1927*a*, *b*, 1929) has stressed the importance of various food materials in deranging or maintaining the efficacy of the gastro-duodenal bactericidal mechanism, particularly in infants. He suggests that such results as those recorded by Webster and Pritchett (1924) and Pritchett (1927) may be due in part to effects of this kind.

We may note here an association between infection and hypersensitiveness to diet, which may prove to be of some importance in the study of resistance.

Coca (1941) has observed in man a syndrome that he ascribes to food allergy, and finds that the incidence of the "common cold" is higher among allergic than among non-allergic human beings. Brown and others (1943) have confirmed his observations.

The Influence of Fatigue on Resistance

In spite of the abundant clinical experience that establishes beyond question the conclusion that bodily rest is an important factor in the treatment of any infective disease, the experimental evidence with regard to the effect of fatigue on resistance to infection is more than a little puzzling.

Spaeth and his colleagues (Oppenheimer and Spaeth 1922, Nicholls and Spaeth 1922, Spaeth 1925) have published a series of observations carried out on rats and guinea-pigs, from which they conclude that the resistance of these animals to tetanus toxin, and to experimental infection with the pneumococcus, is increased as the result of the fatigue induced by making the animals run for a long period in a revolving cage. Bailey (1925) working with rabbits subjected to intratracheal inoculation with pneumococci either before or after exercise, found that severe exhaustion increased their susceptibility to infection. There were indications that repeated intermittent exposures to the fatiguing process rendered the animals refractory to the depressant effect of fatigue. Friedberger and others (1931) have injected rats and guinea-pigs intraperitoneally with El Tor vibrios and have compared the fate of resting animals with that of others fatigued before, or after, or both before and after, the inoculation. There was no significant difference in mortality among the different groups. In the same series of experiments these authors studied the effect of fatigue on antibody production. The results were entirely negative. Such observations must clearly be interpreted conservatively, and without uncritical generalization. They emphasize the stability of the defence mechanisms of the tissues under a variety of different stresses; but they do nothing to lessen the significance of our common experience that fatigue is harmful in established infections, and it would be very unwise to conclude from them that fatigue has no influence on the liability to contract an infection under natural conditions.

More illuminating are the experiments recorded by Boycott and Price-Jones (1926). It may be noted that the infection they studied—that produced by *Salm. enteritidis* in the rat—is a natural disease of that animal, and therefore far more analogous in its pathogenesis to natural human infections than are the experimental conditions selected for study by the workers referred to above. When rats were infected with *Salm. enteritidis* by subcutaneous or intraperitoneal inoculation, fatigue induced before the inoculation, or during the first few days after it, appeared to have very little effect on the subsequent mortality rate. But when the rats were infected by feeding and then fatigued on several occasions during the subsequent few days the results were quite different. Twenty-seven rats were treated in this way and of these 13 died. Twenty control rats were fed on the same cultures, but were not subsequently fatigued; of these none died. The animals that survived the whole period of observation were submitted to necropsy, and it was found, in conformity with the findings recorded by many other observers, that the majority of them were still harbouring *Salm. enteritidis* in the spleen. There is an obvious suggestion that fatigue has a significant effect in activating a latent infection and favouring its fatal generalization.

The Effect of Variations in Temperature, Humidity and Other Physical Conditions of the Environment

The evidence available under this head is concerned mainly with the effect of these environmental factors on the first-line defence mechanisms of the kind considered in Chapter 45. Arnold and his colleagues (Arnold 1927*b*, 1928, 1929,

Arnold and Brody 1927) have studied in considerable detail the effect of high temperature, especially when associated with high humidity, on the gastro-duodenal bactericidal mechanism of the dog. They find that such conditions are associated with a decrease in gastric secretion, a consequent fall in the acidity of the gastric and duodenal contents, and an increased passage of bacteria from the stomach or duodenum to the cæcum.

There are in the literature many other records of experiments on the effect of temperature and humidity on resistance to infection (see McDowell 1923, Kligler and Olitzki 1931, Robertson and Weld 1932, Nishikawa 1935), but the numbers of animals in the comparable groups are so small, and the effects of these two factors seem so difficult to disentangle, that the significance of the results reported cannot be assessed.

Hill and Muecke (1913) have shown that the mucosa of the human nose becomes turgid with blood and covered with a thick, mucous secretion when subjects are exposed to a warm, moist atmosphere, and that when such subjects pass immediately to a cold atmosphere the mucosa pales quickly as the result of a constriction of the vessels, but remains swollen and œdematous for a considerable period. Mudd and his colleagues (1921) have found that chilling of the body surface is associated with a marked fall of temperature in the nasal and post-nasal cavities, and that the return to the normal temperature in these situations is at first rapid, but then very slow, when the body surface is again warmed. There is, then, good evidence of significant changes in the physiological conditions of the nose and nasopharynx in response to changes in temperature and humidity, and it may well be that some of these changes are associated with a lowering of the local resistance; but such a correlation will clearly be very difficult to establish by any statistical survey of naturally occurring infections, and we have as yet no experimental data bearing directly on this point. Nungester and Klepser (1938) demonstrated that exposure to cold increased the aspiration of mucous material into the lungs of white rats; that non-infective doses of pneumococci would infect the lungs of rats when introduced together with mucin; and that though chilling by itself did not lower the resistance of rats to intraperitoneal pneumococci, it greatly increased the incidence of pneumonia in rats inoculated intranasally with mixtures of pneumococci and mucin. They concluded that chilling, by increasing the aspiration of mucous secretions from the upper respiratory tract, might favour the occurrence of pneumonia.

Though they are not concerned with the direct effect of cold, we may note the experiments of Locke (1937) who observed that the more rapidly rabbits recovered their body temperature after artificial chilling, the greater was their resistance to subsequent intravenous and intradermal infection with pneumococci. Here the chilling was employed as an independent test of the physiological response of the animals to an adverse environment. Duerschner, Muschenheim and their colleagues (Hardy *et al.* 1943, Duerschner *et al.* 1943, Muschenheim *et al.* 1943) have recently reported upon the effect of severe chilling in experimental tuberculosis and in pneumococcal infections. Rabbits and guinea-pigs were held under anæsthesia for 6 to 24 hours at 4°–8° C., twice weekly for several weeks. The treatment had no effect on the development of tuberculosis in guinea-pigs, though there was a marked delay in the appearance of skin sensitivity to tuberculin. The response of chilled and normal rabbits to an intradermal lethal dose of virulent pneumococci was similar, except that the local lesions were smaller in the chilled animals. When less virulent pneumococci were injected, chilling resulted in inhibition of the local lesion, and a fatal bacteræmia. The rapid invasion of the blood stream did not appear to be solely due to the absence of a local response, for in other experiments, chilling was found to reduce the minimal lethal dose even of intravenously injected pneumococci.

Among other physical agencies mention may be made of the possible effect of ultra-violet irradiation of the body surface since this procedure has been advocated as a means of increasing resistance to infection. We have noted (p. 1175) that

Colebrook, Eidenow and Hill (1924) have demonstrated a temporary increase in the bactericidal power of the blood as the result of exposure to ultra-violet rays, and a similar effect after exposure to dark heat, or to the blistering of the skin with a mustard plaster. The general experience of the beneficial effect of sunlight as enjoyed under optimal natural conditions, combined with such observations as the above, made it natural to inquire whether any isolated constituent of the solar radiation, and particularly the rays of short wave-length, would produce a significant increase in resistance to intection. Some favourable views have been recorded (Maughan and Smiley 1928, 1929, Hill and Laurie 1931) ; but the balance of evidence appears to be very definitely against the view that exposure to such rays has any significant effect.

Barenberg and Lewis (1928) report entirely negative results in a small group of infants. Colebrook, D. (1929), in a very careful and detailed study carried out on school children of 5–7 years of age, compared the frequency of infection (1) in a group of 101 children irradiated 3 times a week with light from a carbon arc lamp with a high output of ultra-violet rays, (2) in a group of 94 children irradiated under the same conditions except that light of wavelength shorter than 3,200 Å.U. was cut off by a glass screen, and (3) in a group of 92 children who received no irradiation. Over the period of observation—August 1927 to March 1928—the number of colds per 100 child-weeks' exposure to risk was 20·2 in the group irradiated without screening, 18·7 in the group irradiated with the screened lamp, and 18·6 in the control group. The average duration of each cold was 7·5, 7·5 and 7·1 days respectively. The incidence of measles among those exposed to risk and not previously attacked —a small group—showed no significant difference between the treated and untreated: the attack rates were 80·0 per cent. in those irradiated without screening, 70·6 per cent. in those irradiated but screened, and 68·2 per cent. in the unirradiated controls. In the case of whooping cough the attack rates were 8·8 per cent., 7·7 per cent. and 8·6 per cent. In no case did the irradiated group show any benefit. A later report by Doull, Hardy, Clark and Herman (1931) is entirely confirmatory. It deals with a careful study of the frequency of colds among irradiated and unirradiated groups of volunteer students, or members of the staff, at Johns Hopkins University. Every statistical precaution was taken in planning the trial and in assessing its results. These were unequivocal. Among 169 persons in the irradiated group, the attack rate during the period September 29, 1929 to May 31, 1930 was 78·6 per 1,000 student-weeks ; among 184 persons in the unirradiated control group it was 71·9. Whatever test was employed—duration of colds, the presence of fever, the presence of cough or sputum—or whatever allowance was made for previous history or differences in physical conditions, the result was the same: the irradiated group showed no advantage.

The experimental data available are, so far as they go, in accord with field observations. Chapman and Hardy (1931) were unable to obtain any evidence that irradiation increases the resistance of rabbits to the subcutaneous injection of *Pasteurella lepiseptica*. Hill, Greenwood and Topley (1930) found that irradiation conferred no benefit on mice submitted to contact infection with *Pasteurella muriseptica*.

Antibody Formation.—It is convenient at this point to mention recent work on the modification of antibody response. We have already cited a few examples of dietary influences on this function. Scaglione (1938) concluded from a study of rabbits and young children that Vitamin A favoured the agglutinin response to T.A.B. vaccines. Schmeckebier (1943) reports an increase of diphtheria antitoxin in the blood of women given Vitamins A and D. General dietary deficiencies can also modify antibody response. Thus Hartley (1943) found that the substitution of mangolds for cabbage in a basal diet consisting of cabbage, bran and hay, reduced the response of stock guinea-pigs to diphtheria

toxoid (see also Schmidt and Fjord-Nielsen 1934). Cannon, Chase and Wissler (1943) compared the agglutinin response to *Salm. typhi* in normal rabbits and rabbits rendered hypoproteinæmic by partial starvation and by continuous removal of blood plasma and found that the titres of the normal animals at about 8 days were 3 to 5 times greater.

The antibody response in cold-blooded animals like the fish is more effective at high than at low temperatures (see Cushing 1942). Kopeloff and his colleagues observed changes in sheep-cell hæmolysin production in rats after section of the thoracic spinal cord at a high level, but were able to control the variations by maintaining the animals at a constant temperature. Rats kept with their body temperature 5° C. below normal responded poorly to the antigen; raising the temperature to normal was followed by a rise in hæmolysin within 36 hours (Kopeloff and Stanton 1942, Stanton *et al.* 1942). Hartley, Evans and Hartley (1943) investigated the effect of chilling, restricted diet, and infection with *Cl. welchii*, on the secondary response of guinea-pigs to diphtheria toxoid. The experiments were undertaken to estimate the probable effect of adverse conditions of the battlefield on the response of wounded soldiers to " boosting " doses of tetanus and other toxoids (see Chapter 77). Neither a restricted diet, nor daily exposure to temperatures of 2° C. appeared to affect the magnitude of the response to the stimulating dose of toxoid. There was, however, some evidence that the clostridial infection had depressed the response.

Ellingson and Clark (1942) report a series of experiments to test the response of various defence mechanisms to temperatures greater than normal. In rabbits the artificial induction of a severe fever (41·5° C.) slightly reduced the antibody response during immunization with various antigens. It also produced a slight acceleration of the rate of disappearance, and presumably, of destruction of antibodies in the blood stream. Phagocytosis of *Staph. aureus* by human and guinea-pig leucocytes was only slightly depressed at 42° C.; and in rabbits injected intradermally with lethal doses of virulent pneumococci, the maintenance of artificial fever until the infection itself reached the febrile stage made no significant alteration in death rate, survival time or degree of bacteræmia.

Seasonal Variations in Resistance

Seasonal variations in the frequency of particular infective diseases are a commonplace of epidemiology. How far such variations are dependent on fluctuations in the average resistance of the host species, how far on environmental factors that increase the opportunities for the spread of infection, and how far on factors the nature of which we cannot even guess, are problems to which we can as yet give no answer.

Observations are on record from which the conclusion has been drawn that seasonal changes in the efficacy of the normal defence mechanisms do in fact occur (see Pritchett 1925, 1926, Glenny and Waddington 1928, Blake and Okell 1929, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931, Orr, MacLeod and Mackie 1931). Wilson (1930) has noted synchronous fluctuations in the mortalities following the injection of three different strains of *Salm. typhi-murium* into mice during a sixteen months' period, each strain being tested on 15 occasions. It appeared impossible to relate these synchronous changes in mortality to any common factor acting on the three bacterial strains, and it seemed difficult to escape from the view that they must have been due to synchronous transient fluctuations in the resistance of the mice, but these fluctuations showed no definite seasonal distribution.

Even if the occurrence of seasonal variations in resistance were clearly established, it would be an exceedingly difficult task to disentangle the various factors involved; and without such disentanglement there could be little hope of intelligent interference. The vitamin content of foodstuffs, and many other dietetic variables, are subject to seasonal influences; the condition of the nasal mucosa and the

efficiency of the gastro-duodenal bactericidal mechanism are, as we have seen, affected by changes in temperature and in humidity; and there is little doubt that many other types of physiological response, of which as yet we know little or nothing from the immunological viewpoint, may be subject to influences of a similar kind. Until our knowledge of the relative significance of these factors, working in isolation, is much more accurate and detailed than it is at present we can hardly hope to construct a useful working chart of the total effect of the environmental changes associated with the changing seasons.

The Effect of Certain Chemical Substances in Favouring Bacterial Infection

We may include in this chapter a brief survey of a series of observations that have, during recent years, focussed attention on certain chemical substances that play a significant part in the local pathogenesis of infective lesions. It is becoming increasingly clear that some of these factors exert an important influence on the frequency and severity of naturally occurring infections, particularly in industrialized communities.

As a first example we may cite the observations of Bullock (Gye) and Cramer (1919) on the effect of calcium salts in certain anaerobic infections, and the more recent studies of Fildes (Fildes 1927, 1929*a*, *b*, Knight and Fildes 1930, Campbell and Fildes 1931). Bullock and Cramer found that ionizable salts of calcium, inoculated together with the washed spores of *Cl. welchii*, of *Cl. tetani* or of certain other anaerobes, led to the development of the corresponding infections in their most typical and fatal forms, while the washed spores alone showed no tendency to develop in the tissues after inoculation. The chlorides of sodium, potassium, ammonium, strontium or manganese had no such effect. They were unable to determine the exact mechanism by which this effect, to which they gave the name *kataphylaxis*, was brought about; though they were able to show that it was not due to an absence of leucocytes from the lesions, nor to a lack of phagocytic activity on the part of the cells that collected at the site of inoculation. Fildes (1927), attacking the particular problem of experimental tetanus in the guinea-pig, studied the vegetation of spores in the normal testicle, after ligation of the blood vessels. He found that the injection of tetanus spores into the ligated testicle was followed by a rapidly fatal toxæmia, indicating rapid vegetation in the tissues. Comparison of sections from ligated and unligated testicles, at various intervals after the injection of the spores, showed that active vegetation had occurred in the ligated testicle at a period when emigration of leucocytes was at its earliest stage in the unligated testicle, and therefore before any significant degree of phagocytosis could in any case have occurred. He concluded that there must be some definite stimulus to vegetation in tissues cut off from their blood supply, either by ligation of vessels or by the vascular injury following the injection of calcium salts, and suggested that this stimulus was probably the result of the diminished oxygen tension, although Bullock and Cramer had tentatively rejected this view. Later (Fildes 1929*b*) he carried out a detailed study of the oxidation-reduction potential of the tissue fluids under different conditions using suitable indicator dyes. He found that oxidized methylene blue injected into the subcutaneous tissues of the living guinea-pig is not reduced, while reduced methylene blue is rapidly oxidized. In the dead animal the oxidized methylene blue undergoes a slight reduction, while the reduced methylene blue is not oxidized. Analogous results were obtained with thionin and with indophenol I. The general conclusion may be drawn that, in the subcutaneous tissues of the living guinea-pig the oxidation-reduction potential (Eh) at the prevailing pH of about 7·0 is in the neighbourhood of + 0·12 volt, while, as shown in a previous communication (Fildes 1929*a*), tetanus spores will not germinate readily at a potential more oxidizing than + 0·01 volt at this pH (see also Knight and Fildes 1930). In further experiments it was shown that the injection of solutions of calcium chloride

led to the production of localized areas of oxygen deficiency, with an Eh on the negative (reducing) side of that of methylene blue, thus providing conditions suitable for the germination of tetanus spores. Campbell and Fildes (1931) studied the effect of other methods of varying the oxygen pressure of the tissues. Guinea-pigs were maintained in atmospheres containing different partial pressures of oxygen (60 per cent., 20 per cent. and 7 per cent.) and in air containing 0·15 per cent. of carbon monoxide. Control experiments showed the corresponding pressures of oxygen in the subcutaneous tissues (in mm. Hg) to be approximately 26, 20, 5 and 6. When animals subjected to these atmospheres were injected intramuscularly with a mixture of tetanus spores and aleuronat the animals maintained in atmospheric air (20 per cent. oxygen) showed a mortality of 45 per cent., those maintained in an atmosphere of 60 per cent. oxygen a mortality of 6 per cent., those contained in 7 per cent. oxygen, or in air containing 0·15 per cent. CO, a mortality of 75 per cent. These results clearly support the view that the oxygen pressure of the tissues controls the germination of the tetanus spores.

Bullock (Gye) and Cramer (1919) noted that the local defence rupture produced by the injection of calcium salts favoured infection with streptococci, as well as with anaerobic bacilli. The nature of the effect has been studied in more detail by Kettle (1927). Here again there is no evidence of any diminished emigration of leucocytes, or lessened phagocytic activity, and the main effect of the calcium, as revealed by histological examination of the lesions, is a striking increase in the rate of bacterial multiplication. As Kettle points out, the streptococcal-calcium lesions in the mouse bear a close resemblance to the rapidly spreading streptococcal cellulitis that occurs in man. It would appear that the characteristic exudative lesion that is caused by the calcium salt provides peculiarly favourable cultural conditions for streptococci, as it does for anaerobic bacilli; though it does not, of course, follow that the two effects are determined by identical chemical or physical factors.

Calcium is not the only substance which produces defence rupture. That silica has an injurious effect on the lungs and that there is an association between silicosis and tuberculosis has been known for some 30 years, and Gardner (1920) has shown experimentally that a dust containing a high percentage of silica has a significant effect on pulmonary tuberculosis in the guinea-pig. The factors concerned in this enhancement of tuberculous infections by silica have been studied in detail by Kettle (see Gye and Kettle 1922, Kettle 1924, 1926, 1927, 1930, 1932).

The majority of these experiments have been carried out on mice. These animals are normally far more resistant to tuberculosis than is the guinea-pig; and the selective action of silica can be readily demonstrated by subcutaneous injection of the material under test followed by an intravenous injection of living tubercle bacilli. Silica, or silica-containing material, is injected at one spot, some other substance to act as a control at another. If the control substance is an irritant, such as calcium chloride or turpentine, the bacilli that are injected intravenously appear in both the silica and control lesions, but it is in the silica lesion that they show the most active proliferation. The hypothesis of a specific action by the silica is clearly supported by the fact that the more extensive calcium lesion provides far less favourable conditions for the growth of the tubercle bacillus; and by the results of careful comparative tests on a large number of industrial dusts (Kettle 1932), which show that it is the presence of silica, or of a silicate, that determines the characteristic effect. Crystalline silica, unlike colloidal or amorphous silica, is very slowly dissolved in the tissues, and only when dissolved does it exert its injurious action. Silica crystals coated with oxide of iron are not dissolved in the tissues, and they remain inert and harmless.

The pathogenesis of the lung lesions of silicosis, and the association of this condition with tuberculosis, have been studied in numerous experiments in which dusts have been brought in contact with the lung tissue of guinea-pigs or rabbits, either by repeated or

prolonged inhalation, or by the direct injection of a suspension of the dust into the trachea (Kettle 1930).

An interesting experiment is recorded by Gardner (1930). Guinea-pigs were infected, by inhalation, with a strain of tubercle bacillus of unusually low virulence, which normally produces in these animals a retrogressive type of tuberculosis confined to the lungs and bronchial glands. The infection is practically never fatal. When animals so infected were exposed for 8 hours a day to quartz dust an entirely different picture was produced. After 3 to 5 months the bacilli in the lungs began to proliferate actively. The infection spread to all parts of the lungs and to the abdominal viscera, producing large and progressive lesions. The bacilli had not become more virulent, since cultures isolated from the dusted animals produced the usual type of retrogressive tuberculosis when administered to normal undusted guinea-pigs. Vorwald and Delahant (1938) record not only that tuberculosis in the guinea-pig and rat develops with greater vigour in animals treated with silica, but that the immunizing effect of heat-killed tubercle bacilli is inhibited by the simultaneous injection of silica.

Until recently it had been generally assumed that the silica, and the lesions it produced, sensitized the tissues to tuberculous infection. The silica, that is to say, had been regarded as the primary active agent. Kettle (1934) has shown that this view needs considerable modification.

Different groups of guinea-pigs were injected intratracheally with a suspension of silica alone, a suspension of avirulent tubercle bacilli alone, and a mixture of silica and avirulent tubercle bacilli. The dust alone produced a catarrhal reaction, but relatively little fibrosis, and nothing resembling the characteristic nodular silicosis of man. The avirulent tubercle bacilli alone produced relatively little tissue reaction. The mixture of silica and tubercle bacilli produced a characteristic nodular tuberculo-silicosis, closely similar to the human disease. It would seem that the typical silicotic lesions are induced not by silica acting on a normal lung but by the joint action of silica and some bacterial infective agent; and Kettle suggests that the natural human disease should be known as infective silicosis.

Experimental studies of the kind outlined above open up wide fields for further exploration, and we may reasonably hope to obtain light on many problems by studying the ways in which infections may be modified as the result of the local or general action of various chemical agents. Two contributions along these lines may be noted.

Findlay (1928) has attempted to test the tentative hypothesis that the localization of pathogenic bacteria, or of filtrable viruses, at the site of localized tissue injury might be the result of the liberation of histamine, or of histamine-like substances, from the injured tissues, leading to a localized dilatation of the capillaries and an increased permeability of the capillary endothelium. Animals of a susceptible species were inoculated intravenously with an infective agent—the virus of fowl-pox, or of vaccinia, or of the Rous sarcoma, *Staph. aureus*, *Str. hæmolyticus*, or the pneumococcus—and histamine acid phosphate in a 0·5 per cent. solution was then injected into a site suitable for the observation of any lesion, the same amount of phosphate-buffer solution being inoculated into a corresponding site on the opposite side of the body to serve as a control. In each of the experiments the lesions appearing at the site of the histamine injections were significantly more frequent and more severe than those appearing at the site of injection of the phosphate-buffer solution. The latter were indeed infrequent in occurrence and minimal in degree.

Rosher (1931) has studied the effect of the growth-promoting factors (X and V) on experimental infection with *H. influenzæ* in mice. When a suspension of influenza bacilli in ordinary nutrient broth was used for intraperitoneal inoculation, 26 per cent. of 45 strains killed one or more of five mice. When 0·05 ml. of Fildes' solution (containing both

growth factors) was added to the inoculum, 71 per cent. of these strains killed one or more mice.

Effect of Mucin on Bacterial Infection.—Nungester and his colleagues (1932, 1936) noted an exaltation of pathogenicity of pneumococci and streptococci when injected intraperitoneally with a suspension of gastric mucin. Mucin proved to be particularly effective with bacteria which, like meningococci and gonococci, are not naturally pathogenic to laboratory animals, and which were lethal only in doses in which the total amount of bacterial toxins introduced in the infecting dose, not multiplication and spread of the bacteria, was primarily responsible for the death of the animals (Miller 1933, Miller and Castles 1936, Cohen 1936). A similar exaltation of pathogenicity was observed in *H. influenzæ* (Fothergill *et al.* 1937), *Salm. typhi* (Buttle *et al.* 1937), *H. pertussis* (Silverthorne 1938), typhoid bacilli (Rake 1935) and *Staph. aureus* (Anderson and Oag 1939) but not in *Myco. phlei*, " *B. anthracoides*," *Myco. smegmatis*, *Str. viridans* or *Bact. coli* (Anderson and Oag 1939). Substances like agar, starch and gelatin have a similar though less marked effect (Miller and Castles 1936; for negative results with a variety of substances, see Anderson and Oag 1939). The exaltation of pathogenicity produced by the mucin was not accompanied by any demonstrable change in the virulence of the bacteria, but was clearly due to some action on the defence mechanisms of the animal. The efficacy of the mucin has been variously reported as due to its viscosity and power to inhibit intraphagocytic digestion of the bacteria (Nungester *et al.* 1936); to its power to injure the defensive cells and inhibit phagocytosis (Miller and Castles 1936); to its interference with sensitization of the bacteria by antibody (Keefer and Spink 1938). Anderson and Oag (1939) and McLeod (1941) concluded that viscosity alone was not a determining factor, since other more viscous substances were inactive. Activity depended more on the chemical state of the mucin, particularly in the " protein " fraction. According to McLeod, who worked with meningococcal infections, if mucin is to be effective it must be present in adequate amount, and be slowly absorbed from the peritoneal cavity; it promotes infection mainly by permitting the survival and growth of the organism in the peritoneal cavity, but to some extent inhibits the bactericidal action of serum, as well as intraphagocytic digestion, and, if present in granular form, mechanically interferes with the phagocytosis of the bacteria. It may be noted that the viscous, undegraded Group A blood substance isolated from gastric mucin by Morgan and King (1943) behaves like gastric mucin in this respect, and that both viscosity and the power to enhance pathogenicity are lost with the chemical degradation of this substance.

We may conclude this section with reference to benzene and alcohol, two substances that have been employed experimentally to assess the relative importance of the local inflammatory response and of specific antibodies in the defence against infection. In the rabbit, poisoning with benzene reduces or inhibits the local inflammatory response of the skin; there is little hyperæmia, little exudation, and little or no mobilization of leucocytes. More generally, there is an inhibition of leucocyte formation in the bone marrow, and a blood leucopænia. In poisoned animals with no specific immunity, infective organisms (e.g. pneumococci or streptococci) spread rapidly from the site of intradermal injection, and more readily produce fatal generalized infection. Benzene does not apparently interfere with antibody formation, and specific antibodies, whether actively or passively acquired, will to some extent check the spread of bacteria from the local lesion in the absence of leucocytes (Camp and Baumgartner 1915, Hektoen 1916, Rich and McKee 1934, 1939, Schnitzer and Goddard 1943, Schnitzer 1943). Alcohol poisoning also inhibits the normal inflammatory response to local infection. Pickrell (1938) concluded from a study of actively immunized rabbits that the alcohol directly inhibited the reactivity of the capillaries to irritants, thereby preventing a mobilization of leucocytes at the site of a subcutaneous infection with Type I pneumococci. The presence of antibodies in the local inflammatory site was not conspicuously protective in the absence of leucocytes. Lushbaugh (1943) confirmed Pickrell's results in the main, but could not produce a com-

plete suppression of the inflammatory response to intradermally injected pneumococci. Thus the phagocytic response was greatest in the immune, normal rabbit, less in the immune alcoholic, and least in the non-immune alcoholic rabbit. Antibody to some extent protected the alcoholic rabbits, presumably by checking the local infection long enough for the sluggish tissue defences to mobilize.

Nungester and Klepser (1938) demonstrated an increased susceptibility to intranasally injected pneumococci in rats acutely intoxicated with alcohol. They found that the leucocytes of the intoxicated rats had lost their positive chemiotaxis to pneumococci. They concluded, however, that in addition to a generalized depression of the defence mechanisms, the increased susceptibility was probably due in part to the anæsthesia induced by the alcohol, which permitted a readier inhalation of infective material into the lung.

The Influence of Hormones on Resistance to Infection.

There is little doubt that resistance does vary with different states of hormonal balance in the animal body, but the data are for the most part too confused to yield any clear picture of the hormonal effect. The hormonal variations that have received greatest attention in connection with resistance to infection are those of the sex hormones. It is not our purpose to review the literature of sex incidence and mortality in infective disease, nor to consider the variations in resistance that accompany puberty, menstruation, pregnancy and sexual senility; but to give some examples from recent experimental work illustrating the kind of hormonal effects that have been observed.

Schütze, Gorer and Finlayson (1936) record a definite, but inconstantly observed superiority of female mice over male mice in their resistance to infection with *Salm. enteri, tidis* and *Salm. typhi-murium*; but a male superiority in resistance to infection with *Past. muriseptica*. Watson, Wilson and Topley (1938) also noted that female mice were more resistant than males to the epidemic spread of infection with *Salm. typhi-murium*. There are a few experimental studies of the effect of the female sex hormones on resistance. Rosahn, Hu and Pearce (1936) found that male rabbits were less resistant to vaccinia infection than female, and non-pregnant females less resistant than pregnant females. Jungeblut and his colleagues report that poliomyelitis virus was inactivated *in vitro* by a preparation from the urine of pregnant mares and by the serum of the same animals. They were unable to obtain any clear evidence of protection by various hormones in experimental poliomyelitis of the monkey (Jungeblut, Meyer and Engle 1934, Jungeblut and Engle 1934). Aycock (1936) records that œstrin protected 5 of 6 castrated female monkeys against an otherwise fatal dose of poliomyelitis virus, an effect he attributed in part to the thickening and proliferation of the site of injection—the nasal mucosa—under the influence of the hormone. A similar effect may be responsible for the curative action of œstrin in gonorrhœal vaginitis in children. The cells of the immature vaginal mucosa are poor in glycogen; with the onset of puberty, or with the administration of œstrin, they become rich in glycogen, and superficial keratinization occurs. In the œstrin-treated child with vaginitis, the pH of the vaginal contents shifts from about 7·0 to 4·5–6·0, the pus cells and gonococci disappear, and only epithelial cells and Döderlein's bacilli are found (Lewis 1933, Nabarro and Signy 1935, Lewis and Adler 1936; and for later references, see Burpee *et al.* 1939). Apart from the sexual effects of the œstrin, the treatment is on the whole successful. The elimination of the gonococci is attributed in part to the greater resistance of the "mature" keratinized epithelium, and, more generally, to the acidity of the vaginal contents, which is probably the result of lactic acid production by Döderlein's bacillus from the cellular glycogen (see Chapter 90).

In spite of the body of evidence pointing to a modification of the course of syphilis by pregnancy, Kemp (1937) was unable to modify experimental syphilis in rabbits by the administration of œstrogenic hormones. Moderate degrees of protection against

pneumococcal infection were reported by von Haam and Rosenfeld (1942) in white mice of both sexes given injections of œstrone or of testosterone propionate several days previously.

The work of Sprunt and his colleagues provides an interesting example, not only of a hormone effect on resistance, but a relatively simple explanation of how the effect is produced. He observed that the œstrogenic hormone, when injected for a period of 3 weeks into castrated rabbits, decreased the spread of intradermally injected india ink, and increased the resistance of the animals to intradermal vaccinia virus. Pseudopregnant females showed the same phenomenon (Sprunt, McDearman and Raper 1938, Sprunt and McDearman 1940*a*, *b*). The effect appeared to be the reverse of that produced by the Duran-Reynals spreading factor (see Chapter 44) which, when injected with suitable doses of vaccinia virus, increases the degree of skin infection in proportion to the increase in the lateral spread of infective fluids from the injection site. Sprunt (1941) later showed that the interstitial fluid in rabbits was increased by the administration of the hormone, and that the excess fluid in the tissues was responsible both for the failure of vaccinia virus to infect, and of india ink to spread in the skin. More recently Taylor and Sprunt (1943) have reproduced these two effects in rabbits treated by a course of intraperitoneal injections of hypotonic salt solution.

It is clear that we may in the future expect detailed and fruitful analysis of the relations of hormones and resistance. At present these few examples will serve to show the reality of the relation, and some of the ways in which the hormonal effect may possibly be brought about.

The Enhancement of Resistance to Infection by Sulphonamides.

The full discussion in Chapter 6 of the mode of action of sulphonamides on bacteria makes it clear that, of all the hypotheses of the curative action of these drugs, the hypothesis of a direct, *in vivo* antibacterial action has the first claim on our attention. It is in fact widely held that the sulphonamides enable the body defences to eliminate infection by exerting a lethal or bacteriostatic action on the invading organism, or by making it in some way more susceptible to the natural antibacterial defences of the tissues; direct action on the tissues is confined to the toxic effects of overdosage of the drug, and in some cases an interaction of drugs and tissues to yield a different antibacterial substance, as in the breakdown of sulphonamido-crysoidin (Prontosil) to yield active sulphanilamide. The hypothesis is generally acceptable on the grounds that (*a*) the *in vitro* and *in vivo* activity of the various sulphonamides are broadly the same, and (*b*) that the activity of a given sulphonamide varies with the species and often with the strain of the infecting organism. If sulphonamides directly stimulated the body defences, we should expect a greater similarity of action in various infections than is actually the case. The recorded discrepancies between *in vivo* and *in vitro* activity of any one drug and a given bacterium (see, for example, Buttle *et al.* 1937, Gley and Girard 1937) do not necessarily invalidate the hypothesis, for it is seldom possible to make a precise comparison of the concentrations of drugs and drug-antagonizers, and of bacteria, in the test-tube and the tissues, or to assess the contribution made by the tissues to the antibacterial effect.

Various *in vivo* sulphonamide effects on the invading bacteria have been postulated. Capsular degeneration of pneumococci and streptococci was observed by Levaditi and his colleagues (1935*a*, 1939), by Telling and Oliver (1938) and by Whitby (1938), though Long and Bliss (1937*a*), Gay and Clark (1937) and Lyons and Mangiaracine (1938) did not observe it in streptococci, nor Fleming (1938*b*) in pneumococci. Capsular degeneration, it should

experimental *Cl. welchii* and *Cl. septicum* infection, and no synergy in combined drug and antitoxin treatment.

To summarize, the main action of the sulphonamides appears to be directly antibacterial, thereby diminishing the degree of damage produced in the tissues by the infecting bacteria and permitting the more effective action of the natural mechanisms of defence against infection; there is no good evidence of a direct stimulation of the tissue response. Antibody response is modified in so far as the amount or nature of the antigenic stimulus of the invading organisms is altered by the drug; and in certain circumstances, where the tissue responses play a large part in the elimination of infection, the presence of specific antibody appears to enhance the therapeutic effect of the sulphonamides.

The Enhancement of Resistance to Infection by other Substances.

As far as the facts are available, the arguments we have briefly sketched in favour of a purely antibacterial action of the sulphonamides *in vivo*, hold also for the *in vivo* action of the sulphones and other synthetic chemotherapeutic substances, and for penicillin (see p. 184) and other natural antibacterial agents. We cannot exclude the possibility that some of the active substances may prove to be direct stimulants of the tissue defences. It is to be noted, however, that a direct stimulation of this kind is unlikely to be found commonly among substances which, in the current methods of chemotherapeutic research, are selected primarily for their *in vitro* antibacterial properties.

We may note here three recorded examples of protective action by substances not usually regarded as chemotherapeutic.

Wohlfeil (1937) reports that the injection of large prophylactic doses of ferrous salts will protect guinea-pigs against a lethal dose of virulent *C. diphtheriæ*. Treatment after infection prolonged the life of the animals, but did not prevent their ultimate death. The effect was apparently associated with the heavy metallic ion, since sodium and magnesium salts were inactive. The ferrous salts also improved the immune response of guinea-pigs to toxoid and to living bacteria (Wohlfeil and Becker 1938). It seems probable that this effect is analogous to the adjuvant action of other substances in antibody formation, discussed on p. 1115.

The addition of stable emulsions of oil in water to bacterial exotoxins (Walsh and Frazer 1934, Myers 1934, Oerskov and Schmidt 1935) and to cobra venom (Frazer and Stewart 1938) renders them non-toxic on injection into susceptible animals. The detoxication is apparently due to absorption of the toxin at the oil-water interface (Frazer 1936). Frazer and Stewart (1940) noted that mixtures of emulsion and cobra venom were harmless when injected intravenously, but lethal intraperitoneally. It appeared that the emulsion was unstable in the peritoneal cavity, and the venom-interface complex was disrupted with liberation of the toxin, while in the blood stream the serum proteins maintained the stability of the emulsion. It is not yet clear how far the emulsions will protect against or modify established intoxication in the animal.

Finally, simple chemicals may be used to protect susceptible tissues against the entry of an infecting agent. Armstrong (1935, 1936, 1937, Armstrong and Harrison 1936) reported that the nasal instillation of alum or picric acid protected mice against intranasal infection with the St. Louis encephalitis virus, and monkeys against intranasal infection with poliomyelitis virus. Schultz and Gebhardt (1937) found that zinc sulphate was similarly effective against poliomyelitis. The rationale of these procedures is the direct modification of the olfactory area so that it is insusceptible to penetration, or infection, by the virus. The insusceptibility, however, appears to be achieved at the expense of considerable damage to the nasal mucosa (Schultz and Gebhardt 1942, see also Chapter 87).

The Rôle of the Bacteriophage in Infection and Resistance

This is, perhaps, as convenient a place as any to summarize the available evidence with regard to the rôle played by the bacteriophage, or rather by bacteriophages, in infection and resistance. In Chapter 11 we discussed the nature of these lytic agents, and concluded that the balance of evidence was definitely in favour of the view that they are filtrable viruses, parasitic on, or symbiotic with, various species or strains of bacteria. The relationship between the various types of phage and the bacteria that are sensitive to their action is often highly specific; and this specificity seems to be determined, in many cases at least, by the nature of the antigenic components that are situated at the bacterial surface.

The existence of living viruses, specifically attacking various pathogenic bacteria, offered an obvious possibility that they might be employed in the prevention or cure of infective disease. That they can, in fact, be so employed, and are highly effective, has been claimed by d'Herelle (1926); and several observers have reported favourable results in field trials of cholera phage as a prophylactic in India (see Morrison 1932). There are also various observations in the literature with regard to the use of phages as therapeutic reagents in human infections (see Krueger and Scribner 1941); but these are discrepant, and not of the kind that provide us with the evidence we need, though on the occasions when large-scale observations are made with as great a care as the circumstances permit, the results are not encouraging (see Boyd and Portnoy 1944). Moreover, aside from a theoretical lethal action of the phage on the infecting bacteria, it is possible that the phage filtrates are beneficial because they act as specific vaccines, since they contain the antigens of lysed bacteria; as stimulants of a non-specific immunity; or as opsonins (see Krueger and Scribner 1941). In this last connection we may note that MacNeal, McRae and Colmers (1938) demonstrated a marked opsonic effect when optimal quantities of specific phage were mixed with *Staph. aureus* and added to human blood. Until some years ago there was on the experimental side little evidence that phage had an *in vivo* antibacterial action, excepting a few observations suggesting that phages injected into an animal simultaneously with such organisms as *Salm. typhi* or *Bact. coli*, had a slight protective action (Wollman 1925, Arnold and Weiss 1926, Walker 1929).

Thus there was a general failure to demonstrate any protective or curative action of phage filtrates against *Salm. typhi-murium* or *Salm. enteritidis* when tested in mice (Topley and Wilson 1925, Topley *et al.* 1925, Levy 1925, Wollman 1925, Richet and Hauduroy 1925, Bronfenbrenner and Korb 1925–26, Ebert and Peretz 1929, Greenwood *et al.* 1936). Similarly, phage filtrates had little or no protective or curative action in salmonella infections in fowls (Pyle 1926), in experimental plague in rats (Compton 1928, 1930, Doorenbos 1929), in hæmolytic streptococcal infections in rabbits (Clark and Clark 1927) or in guinea-pigs (Colvin 1932), or in experimental anthrax in the mouse (Cowles and Hale 1931). Nor was there any experimental evidence that the presence in the intestines of mice of a phage acting on *Salm. typhi-murium* prevents the spread of natural contact infection with that organism (Topley and Wilson 1925, Topley *et al.* 1925, Greenwood *et al.* 1936).

In 1937, Asheshov, Wilson and Topley provided clear-cut evidence of a protective and to some extent a curative action of phage. A mixture of $1{\cdot}5 \times 10^9$ particles of a Vi phage, and 10^8 living virulent typhoid bacilli of the Vi type was held for 10 minutes *in vitro*, and proved innocuous on intraperitoneal injection into

mice. The protective effect was specific, and depended on the presence of active Vi phage. Intravenous phage was also fully protective against intraperitoneal bacilli, when given at the same time; it was protective when given two, but not four, hours after infection.

These results have been confirmed by Fisk (1938), Yen and Chang (1941), Pio de Roda and Saqueton (1941) and Ward (1943). Asheshov and his colleagues pointed out that the typhoid infection in their mice was a highly artificial disease, the lethal dose of bacilli being large and producing an acute toxæmic death. Ward overcame this objection to some extent by using mucin to enhance the virulence of the typhoid bacilli, so that 10–100 organisms were lethal. Intravenous phage, 2 hours after infection, proved highly protective against this type of infection. The protection did not appear to be due to stimulation of phagocytosis, nor to any immunizing power of the phage filtrate. There was some evidence that actual *in vivo* lysis of the infecting bacteria occurred, and a marked increase of circulating phage occurred some hours after its injection. The healthy survivors among the treated mice carried the typhoid bacilli for some weeks, but many of the organisms on isolation proved to be degraded variants without Vi antigen. That the protective action of phage is accompanied by its apparent multiplication *in vivo* is clear from the experiments of Rakieten and Rakieten (1943) and of Dubos and his colleagues (1943). The former protected 10-day-old chick embryos from a fatal infection with *Sh. flexneri* by a single dose of specific phage, and found an association between survival of the embryos and a demonstrable increase in their phage content. The latter injected Shiga dysentery phage into the peritoneal cavity of normal mice, and found that the phage content of the tissue rose to a maximum in 2 hours and then declined rapidly; the phage content of the brain was much lower than that of the blood. When the phage was injected into mice inoculated intracerebrally with *Sh. shigæ*, the phage content of the brain increased rapidly till it exceeded that of the blood, and reached a maximum in 12 hours. At the same time the mice were protected against an otherwise fatal meningitis. Oral phage afforded no protection.

These results have been achieved by the administration of very large numbers of phage particles, and in special experimental circumstances. Their applicability to the therapy of natural infective disease is still to be determined, and the problem of the great discrepancy between the high *in vitro* and the low *in vivo* activity of a given phage is still unsolved.

One possible reason for the failure of therapy is the development in the tissues of phage-resistant variants of the infecting bacterium (see Chapter 11). In the experiments cited above, both Ward (1943) and Rakieten and Rakieten (1943) noted the presence in phage-treated tissues of variants that were presumably induced by the action of phage, but they appeared to have little virulence. On the other hand, strains of *Salm. typhi-murium*, isolated from mice that have died of infection in spite of the presence of phage in the tissues, have proved just as sensitive to that phage as the bacteria originally inoculated (Topley and Wilson 1925 Topley *et al.* 1925, Greenwood *et al.* 1936).

Blood, serum, bile, white cells, tissue debris can all inhibit lytic action of phage *in vitro* (see Evans 1933, Applebaum and Patterson 1936, Zaytzeff-Jern and Meleney 1936). Little, however, is known of the mechanism of phage inhibition. It may be (see Zdansky 1924) that the cells or colloids of the body fluid exert *in vivo* an inhibiting effect on phage lysis similar to the inhibition exerted *in vitro* by high concentrations of gelatin or agar (Bail 1922, Doerr and Berger 1922, Otto and Munter 1923, Bronfenbrenner and Korb 1925). This suggestion is supported by the observation that hitherto the most hopeful results of bacteriophage therapy

have been obtained in cholera—a disease in which the infecting vibrios are multiplying freely in the watery contents of the intestine.

In any case, until we know more than we do of the factors that prevent any but high concentrations of phage from exerting any protective effect in the tissues, we cannot justifiably conclude that it is impossible so to adjust the conditions that a large part of the *in vitro* activity might be made available for therapy in the infected animal.

SUMMARY

(1) With regard to the effect on resistance of variations in diet, the one fact that stands out clearly is that a gross deficiency in the intake of Vitamin A, especially during the period of rapid bodily growth, is associated with a marked increase in susceptibility to infection. There are suggestions that C-avitaminosis, in animals peculiarly liable to this condition, may be associated with a similar break in resistance; and that resistance can be to some extent restored by the administration of the vitamin; but the evidence is highly confusing. There is little consistent evidence that the *in vitro* antibacterial and antitoxic action of the vitamin is reflected in a prophylactic action of excess vitamin against infection or intoxication. In certain cases resistance is altered by deficiency of one or other of the B vitamins, sometimes in the direction of increased susceptibility, and, in certain virus diseases, in the direction of increased resistance. There is no good evidence that a deficiency of Vitamin D has any significant effect on resistance. There is little if any evidence that an excess of Vitamin A, above the limit required to prevent the occurrence of detectable avitaminosis, has any prophylactic or therapeutic effect in infective disease, with the possible exception that some prophylactic effect may be exerted by the administration of Vitamin A during the late stages of pregnancy.

(2) Our knowledge of the influence of other dietetic factors on resistance to infection is as yet too scanty to justify any general conclusions. Recent observations show that certain food substances, like dried milk, when added to an otherwise adequate diet increase the resistance of mice to certain bacterial toxins, and certain bacterial infections; and it seems clear that this is not a direct vitamin effect. Studies of this kind are, however, in their infancy.

(3) In general, it is only the dietary deficiencies, whether of vitamins or foodstuffs, of a degree leading to manifest deterioration of the general bodily health, which are unequivocally associated with depression of resistance to infection, and inhibition of the cellular and antibody responses to the invading organisms.

(4) The experimental evidence with regard to the influence of fatigue on resistance suggests that it is more important as a factor leading to the activation of a latent infection than as predisposing to infection *ab initio*.

(5) The data with regard to the effect of variations in the physical environment are scanty. It is certain that fluctuations in temperature and humidity affect the local conditions in the upper part of the respiratory tract, and it seems possible that the changes produced may in some cases lower the local resistance. There is evidence that similar environmental changes may derange the gastro-duodenal bactericidal mechanism. Prolonged exposure to cold appears to depress the defensive tissue responses to infection. There is no satisfactory evidence that irradiation with ultra-violet rays produces any significant increase in resistance to natural or experimental infection.

(6) There are suggestions that a seasonal variation may occur in the general resistance to infection. If this be the case, such fluctuations are probably dependent on variations considered in the previous sections; but which factors are concerned or what their relative importance may be we cannot tell.

(7) Evidence is accumulating with regard to the importance of certain chemical substances as agents favouring local infective processes. Thus ionizable calcium salts favour the germination of tetanus spores in the tissues, and this fact is of importance in aiding the natural pathogenesis of tetanus in wounds grossly contaminated with soil. Silica favours the local proliferation of the tubercle bacillus in the tissues, and is an important predisposing cause to this infection in certain industrial populations. The mechanisms upon which these kataphylactic effects depend have been subjected to detailed study with results of considerable interest and importance. Chemotherapeutic agents like the sulphonamides, and natural antibacterial substances like penicillin, do not directly stimulate the defence mechanisms of the infected animal. Their action is predominantly if not exclusively antibacterial. Nevertheless, their total therapeutic effect depends to a substantial though varying degree upon the natural cellular and humoral antibacterial responses of the tissues.

(8) Under controlled experimental conditions, bacteriophages have been shown to be both protective and curative *in vivo*. There is as yet little evidence of successful phage therapy of natural infections, except perhaps in cholera.

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CHAPTER 55

IMMUNITY IN VIRUS DISEASES

THE virus diseases of man and animals are described in Chapters 85 to 89, and that description includes the immunity reactions that occur in each particular infection. It is, however, even at the cost of some repetition, desirable to summarize and discuss the available data with regard to immunity to virus diseases as a whole, as contrasted with immunity to bacterial infections. There was a tendency among earlier workers to differentiate somewhat sharply between immunity to these two classes of parasite ; and this differentiation involved, mainly by assumption, the mechanisms brought into play as well as the quality and duration of the immunity induced. The problem that mainly concerns us here is the extent to which this differentiation is justified. Almost all experiments on the transmission of virus infections, and on immunity reactions *in vivo* or *in vitro*, have of necessity been carried out with tissue extracts or with other crude material containing host products as well as virus. Under these conditions the qualitative or quantitative analysis of such reactions is rendered very difficult, and for this reason the study of antiviral immunity has tended to lag behind that of antibacterial immunity at just those points in which the latter has become most precise. We have, however, learned enough of the mechanisms concerned to construct a useful working outline which can be filled in rapidly when our technical difficulties have been overcome, and which has already passed the sound empirical test of enabling us to interfere with considerable success in the natural propagation of infection.

Antiviral Immunity as Observed under Natural Conditions. Latent Infections.

The first fact that forces itself on our attention when we examine the natural history of virus diseases is the frequency with which an effective and lasting immunity is afforded by a single attack (see Andrewes 1931). Smallpox, chickenpox, measles, mumps, cattle plague, swine fever, and dog distemper—all these are common virus diseases, and all confer an immunity that, in the great majority of cases, appears to last throughout life. There are exceptions. Herpes simplex is one ; influenza and the common cold are others. Not long ago foot-and-mouth disease would have been placed in this non-immunizing or poorly immunizing class ; but we have had to reconsider our views in that regard, and perhaps the relations that have misled us in this instance have misled us in others also. It is well to remember, as Bedson (1937) points out in his valuable review, that the mechanism of immunity probably varies with different types of viruses, as it does with different types of bacteria, and that generalizations about the similarity or difference of immunity in bacterial and virus diseases are apt to be misleading.

Though, at one time, the idea was prevalent that infection with a pathogenic

virus invariably resulted in a frank case of the disease, diagnosable by ordinary clinical methods, it has become increasingly clear of late years that atypical, abortive, and latent infections are very common and play an important part in the genesis of naturally acquired immunity. Even in so highly contagious a disease as measles, there is evidence to show that not every susceptible child who is exposed to infection contracts the disease.

Stocks (1928, 1930*a*, *b*), in a series of careful analyses of the relevant data, has shown that the morbidity figures for measles, for chicken-pox and for German measles are inexplicable except on the assumption of immunization in the absence of diagnosed disease, or of an effective inherited immunity. That the former factor is operative can be demonstrated by comparing, during any epidemic prevalence, the attack rate among children who have previously been intimately exposed to risk without contracting the disease with that among children who have previously been less intimately exposed. The natural history of these widely prevalent virus diseases, as developed by Stocks, presents striking analogies to the natural history of diphtheria or of scarlet fever.

In other virus diseases the occurrence of atypical cases and of healthy carriers has obtained general recognition. Wickman (1907), for example, pointed out that it was impossible to account for the epidemiological behaviour of poliomyelitis without assuming the existence of latent and atypical infections; and Stocks (1932), examining the available data of several outbreaks, calculates that the ratio of those developing latent immunizing infections to those developing clinical attacks of the disease is probably of the order of 100 to 1 or more.

In support of the epidemiological evidence is the finding that protective antibodies acting on the poliomyelitis virus are widely distributed among the population at large.

Aycock and Kramer (1930*a*, *b*, *c*), using the highly susceptible monkey as a test animal, determined the protective power of samples of serum obtained from convalescent cases and from normal persons. Some 90 per cent. of the former and just over 50 per cent. of the latter showed protective antibodies in the serum. Similar tests were later carried out on normal persons, giving no history of poliomyelitis, living in another area of the United States. Of 21 adults, 18 showed neutralizing antibodies in their blood. Brodie (1932) has recorded similar findings in Montreal.

If we accept the view that the presence of such antibodies indicates previous contact with the virus of poliomyelitis we can hardly escape the corollary that mild atypical attacks, or a purely immunizing carrier infection, must be an exceedingly common event in this particular disease. Some observers, however, believe that protective substances occur in the serum of normal persons apart altogether from any specific stimulus (see Jungeblut and Engle 1932*a*, *b*, 1933, 1934, Jungeblut 1933, 1934). The problem is analogous to that of the origin of the natural antibacterial antibodies (see Chapter 49).

In addition, there are now several reports of the isolation of poliomyelitis virus from the nasal secretions or fæces of healthy carriers (Flexner *et al.* 1913, Kling and Pettersson 1914, Lépine *et al.* 1939, Kramer *et al.* 1939, Langmuir 1942).

Yellow fever is another disease in which latent infections appear to be very common. Serum protection tests in South America and West Africa have shown that in endemic areas, where clinical cases are infrequent and sporadic, a high proportion of the children show neutralizing bodies in their blood, indicating that the overt cases of disease give a quite erroneous picture of the real frequency of infection (Beeuwkes *et al.* 1930, Russell 1932, Hughes and Sawyer 1932, Soper *et al.* 1932, Mahaffy *et al.* 1933, Beeuwkes and Mahaffy 1934).

The degree of latency may be even more striking.

In lymphatic choriomeningitis of mice, for example, infection of every mouse in a particular stock occurs, either *in utero* or soon after birth, giving rise to few or no symptoms, and remaining latent for the rest of the animal's life. Antibodies are not usually developed, and the persistence of the virus can readily be demonstrated by inoculation of tissue extracts into mice of a non-infected stock (Traub 1936*a*, *b*, 1938, 1939). In this instance an almost perfect equilibrium appears to be established between the virus and its host.

In Virus III infection of rabbits, and in some of the mouse pneumonia virus infections, a similar state exists, but the presence of the virus can be brought to light by repeated passage through normal or infected animals, the virus ending by giving rise to characteristic disease in the host (see Andrewes 1939).

So far, we have been considering latent infections in healthy persons or animals. Attention must now be drawn to the persistence of virus in convalescent patients who have suffered from a typical or atypical form of the disease in question. Here again the degree of latency varies to some extent.

Herpes febrilis virus, for instance, often affects young children, giving rise to stomatitis. Such patients are liable to recurrent attacks throughout life of herpes labialis, particularly in association with colds, pneumonia, and certain fevers. The virus appears to lie latent in the tissues between attacks, and to flare up on receipt of the appropriate stimulus. Antibodies are demonstrable in the blood, but they have apparently little or no effect in preventing a relapse (Dodd *et al.* 1938, Burnet and Lush 1939*a*). In poliomyelitis and yellow fever, on the other hand, the persistence of the virus is associated, as a rule, with a solid immunity. The poliomyelitis virus has been isolated from convalescents several months after the disease; and in yellow fever the probable persistence of the virus may be conjectured from the finding of antibodies in patients many years after an attack of the disease, even when no contact with the active agent has occurred in the meantime (see Rivers 1943). Psittacosis in experimentally infected mice, and infectious anæmia of horses, are two other examples in which the virus seems to remain indefinitely in the tissues after it has given rise to an attack of the disease. In neither of these infections are neutralizing antibodies evident to any extent, and it is easy therefore to demonstrate the presence of the virus by inoculation of a tissue suspension or a drop of blood into a susceptible animal.

There is strong reason to believe that in these and many other virus diseases the continuance of a solid immunity is dependent upon persistence of the virus in susceptible cells of the body. The transient nature of the immunity that develops towards virus diseases of the respiratory tract, such as the common cold and influenza, may perhaps be due to the desquamation of the surface cells of the mucosa, which cannot therefore afford a permanent home for the infecting viruses (Rivers 1943).

The Mechanism of Virus Infections.

It would appear that the general process of tissue invasion in virus diseases does not differ in any essential way from that described in the case of bacterial infections. There would seem, in many cases at least, to be the same sequence of local proliferation, blood-stream invasion and secondary foci of infection, each phase varying in prominence according to the virulence of the virus and the susceptibility or resistance of the host.

Thus, Todd and White (1914) note that, when cattle are injected subcutaneously with a small dose of the virus of rinderpest, the blood usually remains non-infective for 72 hours, becoming infective coincidently with the onset of illness; and Andrews and others (Report

1931) note that the virus of foot-and-mouth disease does not appear in the blood of experimentally infected cattle until shortly before the commencement of the febrile reaction, though it may be present in high concentration some hours before the temperature begins to rise, or before vesicles appear. The virus then tends to persist in the blood throughout the febrile period, though the degree and persistence of blood-stream infection varies widely in different animals (see also Waldmann, Trautwein and Pyl 1931). In the case of yellow fever, Hudson and Philip (1929) record that monkeys bitten by infected mosquitoes show the presence of virus in their blood 1 to 2 days after infection, and at about the same interval before the onset of fever.

Vaccinia, as regards its obvious lesions, is commonly limited to the site of inoculation into the skin ; though the virus may be recovered from the blood after intradermal inoculation in the rabbit (Ohtawara 1922, Rivers and Tillett 1923, Gildemeister and Heuer 1927), and Gins and his colleagues (1929) have reported its presence in the nasal secretion of children after ordinary cutaneous vaccination. With highly potent strains of virus a generalized vaccinia may occur after dermal inoculation in the rabbit, with a widespread eruption, pocks on the lips and tongue, and lesions in the lungs, liver and other organs (Douglas, Smith and Price 1929). When such a virus is injected intravenously this severe, and sometimes fatal, generalization occurs with considerable frequency, and there is a rough proportionality between the dose of the virus administered and the degree of resultant generalization. When less potent viruses are injected intravenously there is frequently some degree of generalization, evidenced by an eruption of pocks on previously shaved areas of skin or on the mucosa (Calmette and Guérin 1901, Camus 1917–18, Levaditi and Nicolau 1923).

On the other hand, in the more strictly nervous diseases, such as rabies, there is no evidence to suggest that local proliferation or blood-stream invasion occurs ; the virus appears to gain access to the central nervous system by way of the axis cylinders, and to diffuse through the brain and cord entirely by nervous paths. There is no exact analogy to this behaviour in the diseases caused by bacteria, though the route of diffusion appears to be closely paralleled by tetanus toxin.

Many viruses have naturally a strong selective affinity for certain tissues, such as the vaccinia virus for the cells of the epidermis and the poliomyelitis virus for the anterior horn cells of the cord. It is customary to speak of such viruses as *dermotropic* and *neurotropic* respectively. Others, such as the yellow fever virus, which affects particularly the liver, are sometimes spoken of as *viscerotropic* ; and others, such as the psittacosis virus, which affect tissues widely distributed throughout the body, as *pantropic*. All these terms are relative and are easily abused. In general, they are of more value when referring to the properties of different strains of the same virus than in comparing different viruses. By passage experiments and other means variants of a given strain may be developed that show considerable differences in their tissue localization and in their pathogenicity. Examples are the street virus and the fixed virus of rabies, and the viscerotropic and the neurotropic variants of the yellow fever virus.

The histopathology of the virus diseases, though differing in some respects from that of most bacterial infections, does not differ so sharply and fundamentally as to require a separate generic description. There is only one characteristic peculiar to the viruses, namely their ability to form *inclusion bodies* in the cells they invade. Even this is essentially similar to the intracellular multiplication of such an organism as the leprosy bacillus, the resulting colonies differing in no material way from the virus colonies of which the cytoplasmic inclusion bodies are composed. The tissue lesions that are associated with virus infections—inflammatory, necrotic, degenerative, granulomatous and proliferative—are of the same kind as those that

follow infection with one or other of the pathogenic bacteria ; and the stress of the reparative reaction appears to fall on the same cells of the reticulo-endothelial system (see Ledingham 1924, 1926*a*, *b*, 1927, Ledingham and Barratt 1929).

Of the ultimate mechanisms by which the filtrable viruses produce their harmful effects we as yet know little or nothing. We do not, for instance, know whether any of them produce soluble toxins with a characteristic pharmacological action, such as are produced by certain bacteria. Until our technique has developed to the point at which we can secure mass cultivation *in vitro*, in a cell-free medium, this problem is likely to remain unanswered. We can at the moment go no further than the statement that no instance has as yet obtruded itself upon our notice in which it seems necessary to assume the presence of a soluble toxin acting at a distance from any focus of living virus. On the other hand, there is some evidence to suggest that endotoxins, similar to those contained in certain bacteria, may be produced by viruses. Rake and Jones (1944), for example, have found that the lymphogranuloma, meningopneumonitis, and mouse pneumonia viruses (see Chapter 85), when grown in the yolk sac of the developing chick embryo, may elaborate a thermolabile endotoxin, which is capable on intravenous inoculation into mice of causing death in 4 to 24 hours.

Antiviral Immunity.

Before discussing the mechanism of antiviral immunity it will be convenient to consider briefly the ways in which such immunity may be induced ; since it is held by many that, in this respect, there is a significant difference between the behaviour of the filtrable viruses and bacterial cells.

Active Antiviral Immunity.—As regards active immunity, we have seen that this is conferred under natural conditions by a typical attack of the disease, and probably by atypical attacks of varying severity or by a carrier infection. Artificially it may be induced by the injection of a sublethal dose of living virulent virus (a dangerous and uncertain method, even when injections are made by an unusual route), by the injection of a virus that has changed its disease-producing characters, but not its antigenic structure, during adaptation to a different host-species—the classical example is the use of vaccinia to protect against variola—or by an initial injection of a fully attenuated virus followed by injections of less and less attenuated samples until the fully virulent virus is resisted—the classical example is the Pasteurian method of protection against rabies by the injection of rabbit-cord virus attenuated by drying. The question at issue is whether we can induce an effective antiviral immunity by the injection of killed virus alone, in the way that we can produce an antibacterial immunity by the injection of killed bacterial cells. The question really resolves itself into two others. Can we produce an effective resistance by the injection of virus-containing material so completely inactivated that it produces no detectable infection when injected in large doses into a fully susceptible animal ? If this is possible, may we assume that the inactivated virus is actually dead ?

We may answer the first question affirmatively, provided we qualify it by saying that the immunity produced by an inactivated virus is seldom of long duration, unless it is followed up by a later injection of active virus.

Semple (1912) has shown that the classical Pasteurian methods of rabies prophylaxis may be replaced by injections of rabbit-brain virus inactivated by treatment with 1 per cent. phenol. Boynton (1918, 1928) records successful immunization against cattle plague with

a phenolized vaccine, Curasson and Delpy (1926) and Daubney (1928) with a vaccine inactivated with formol. Ducloux and Cordier (1926) report successful immunization against sheep-pox with vaccines inactivated by formol or by ethanol. Bedson, Maitland and Burbury (1927) record the partial immunization of guinea-pigs against foot-and-mouth virus by the injection of formolized virus, and the complete immunization of a proportion of animals after prolonged treatment. Todd (1928*b*), having failed to immunize fowls by the injection of heat-killed or formolized fowl plague virus, succeeded in producing an effective immunity by the injection of three doses of a phenolized vaccine. Bedson (1931) produced a high degree of immunity in guinea-pigs against herpes by the injection of a formolized virus and records similar results with inactivated psittacosis virus (see Discussion 1932). Other examples will be found in vaccines against distemper, Rift Valley fever, equine encephalomyelitis, and influenza.

It would seem a fair conclusion that the injection of inactivated virus material almost always confers some degree of specific resistance, provided that a suitable method of inactivation is employed; but that the resistance so conferred varies widely in effectiveness in different virus diseases. In some it is of a high order. In others it must be reinforced by injections of active virus; and it is probable that such reinforcement always increases the solidity and duration of the resistant state.

When we inquire whether an inactivated virus may be assumed to be a dead virus, we are on more difficult ground. There is general agreement that the application of violent, and therefore certain, methods of killing—such as heating to high temperatures for long periods—destroys the antigenic value of virus-containing material. Indeed, as we have seen above, inactivation by such a chemical agent as formol may remove the antigenic value of one virus but not of another. Such facts are, perhaps, in favour of the view that attenuation rather than death marks the limit beyond which we cannot pass if an effective antigenic stimulus is to be obtained; but they are also explicable on the assumption that the lack of immunizing effect is due to some alteration in an important antigenic constituent rather than to the killing of the virus. If all effective vaccines were attenuated, not killed, we might expect occasional infections with our treated vaccines. In some instances at least such accidents seem never to occur. Thus (see Harvey and McKendrick 1930), the testing of 500 samples of Semple's phenolized rabies vaccine by the injection of 1 ml. intramuscularly into rabbits did not result in a single infection. Similarly, if a symptomless infection occurred in an animal injected with an inactivated vaccine, it might be possible by passage from that animal through others to produce characteristic symptoms or lesions. Bedson (1931) notes that his formolized herpes vaccine produced no lesions when introduced into the plantar skin of guinea-pigs, nor was it possible by passage from animal to animal by this route to restore its activity. Even these results are not entirely convincing, since it might plausibly be maintained that the chemical treatment had affected the virulence of the virus without destroying its vitality. Failure to grow in tissue culture or in the developing chick embryo might be a more convincing test. Taking the evidence as a whole, however, we may agree with Bedson (1937) in regarding, for all practical purposes, such inactivated viruses as dead. But when we look more closely into our terms we are faced with the difficulty of defining "living" and "non-living" in relation to viruses. This is a question that need not detain us here; it has already been discussed in Chapter 41 (see p. 967).

Whatever the final verdict may be, it is the common practice at the moment to rely on the injection of living virus under the protection either of a partial active immunity induced by the previous injection of virus inactivated in some appropriate way, or of a temporary passive protection induced by the injection of serum from an immunized animal. The latter method is very commonly employed, and its practical exploitation has led to some interesting observations. These have been admirably summarized by Andrewes (1931).

Virus-serum mixtures incubated until infection can no longer be produced by any route seem to be useless as immunizing agents. To be effective they must be slightly under-neutralized. In this state they will produce immunity against some virus diseases. A more generally effective method is the so-called side-to-side inoculation—virus into one site, serum into another. Depending upon the ratio of antiserum to virus, this procedure may be followed by a mild and atypical infection or may produce no detectable general reaction. In some cases effective immunity is secured only if the serum-virus ratio is within the range at which a generalized reaction occurs; in others, such as dog distemper (Laidlaw and Dunkin 1931), an excess of antiserum sufficient to suppress such a reaction does not prevent a successful immunizing response. Side-to-side immunization has been successfully employed in cattle plague (Kolle and Turner 1898, see also Carmichael 1928), swine fever (Dorset *et al.* 1919) and dog distemper (Laidlaw and Dunkin 1931). Theiler (1907-1909) records the successful immunization of horses and mules against African horse sickness by the simultaneous intravenous injection of virus and antiserum.

Another effective method is to administer the antiserum after the virus. This involves a mild immunizing attack of the disease, and for this reason it will clearly never be a method of choice where times and conditions may be determined at will; but it has an important application when exposure to risk of infection has already occurred, or is an imminent and unavoidable danger. By the administration of antiserum in adequate amount, and at the correct interval after exposure, it is possible to avoid a severe and typical attack, replacing it by a trivial infection that will confer a lasting immunity. The modern methods of measles prophylaxis (see Chapter 88) afford an excellent example of successful action along these lines.

Passive Antiviral Immunity.—Turning to passive immunity, enough has already been said to indicate that antiviral sera are at least as effective in affording protection against the homologous virus as is an antibacterial serum in protecting against the homologous bacterium. It may be noted that, while antibacterial sera are commonly prepared by the immunization of some conveniently large animal, usually the horse, it has become the practice to prepare antiviral sera by the immunization of an animal of the same species as those it is desired to protect. This is in part due to the fact that much of the work on virus diseases has been carried out within the province of veterinary medicine, and that the original observations on the protective power of immune sera were made with the blood obtained from convalescent animals, or from such animals after further treatment with large injections of active virus. These hyperimmunized animals, when of a conveniently large kind, provide a supply of highly effective antiserum. There are, moreover, many observations which suggest that homologous antisera may be more effective than those prepared in animals of another species. Thus an antidistemper serum prepared in the dog will protect both dogs and ferrets against distemper virus, but protection in the ferret is much less effective and less consistent than in the dog (Laidlaw and Dunkin 1931). [Cf. the efficacy of different antisera in passive anaphylaxis, p. 1140.]

As in antibacterial immunity, the passive immunity so obtained is transient—too transient to be of any prophylactic value except under narrowly limited conditions that only occasionally occur in practice.

There are observations on record—some of them contained in the papers already referred to—indicating that antiviral sera may have a definite if limited therapeutic value, though there is reason to believe that once virus has gained access to the cell no circulating antibody, whether generated actively by the host or passively introduced, is able to neutralize it.

The Mechanisms of Antiviral Immunity.

We know very little of what happens when a virus is introduced into the tissues of a resistant animal, or when a relatively avirulent strain is injected into a relatively susceptible animal. We cannot, therefore, construct any detailed pictures of the kind outlined in Chapter 47 in the case of bacterial infections.

Our ignorance on this point is due to technical difficulties rather than to lack of interest. In the case of bacterial infections we can, by staining and by cultivation, trace the distribution of the parasites in the tissues with considerable accuracy. In virus diseases neither method is available in a form suitable for this purpose, and we are thrown back on the inoculation of a susceptible animal whenever we wish to demonstrate the presence of virus in a given tissue. If we desire our results to be quantitative—as we usually do—we are in further difficulties unless the virus produces a characteristic skin reaction. If it does, we can titrate it with some approach to accuracy. If it does not we have to rely on producing infection or death in an adequate sample of animals with each dose tested, and our task becomes formidable. Nor do our troubles end here. The material we inject contains not only virus, but products derived from the host; and the possible effects of the latter cannot be neglected. The assumption that the result obtained by the injection of virus-containing material into a susceptible animal is determined by the virus alone is, as we shall see, quite unjustified.

Such knowledge as we have has been gained mainly by experiments on normal animals. So far as it goes it suggests that the immediate clearing mechanism involved in freeing the blood stream and tissue fluids from virus is the same as that brought into play in the case of bacteria, and that, in a naturally immune animal, or in a relatively susceptible animal injected by a route that does not allow the virus to gain easy access to the tissues for which it has a special affinity, this clearing mechanism is capable of functioning very effectively.

Thus Gins and Weber (1916) found, in conformity with the results of earlier workers, that vaccinia virus of ordinary potency disappeared rapidly from the blood stream after intravenous injection. When large amounts of virus were injected, and the organs were tested within 5 hours, virus could usually be recovered from the spleen, less frequently from the liver, but never from the bone-marrow. Douglas, Smith and Price (1929)—who were, it will be remembered, working with a highly potent strain of virus—record four experiments in which various organs were tested for virus within a few days after an intravenous injection. In these the virus was recovered in each case from lungs and spleen, in 3 of 4 cases from the liver, and in 3 of 4 cases from the bone-marrow; but the fact that infection was in all cases widely generalized makes the significance of these findings doubtful. Of more interest are the relative frequencies of vaccinial lesions in the different organs and tissues. Of 36 rabbits injected intravenously the numbers showing lesions at different sites were as follows: skin 31, mucosa of lips or nose 26, tongue 24, lungs 35, liver 24, spleen 24, adrenals 14, œsophagus 1, bladder 1, intestine 1, muscles 2, heart 1, pericardium 1, peritoneum 3. Lesions were also relatively frequent in the testes among the males, and in the ovaries among the females. Thus we find the highest frequencies in those tissues for which vaccinia virus is known to have a special affinity and in those organs—lungs, liver, spleen and adrenals—which are primarily concerned in freeing the blood stream from inanimate foreign particles or from bacteria.

Flexner and Amoss (1914) record that the injection of poliomyelitis virus into the veins of monkeys—a route that does not commonly lead to the production of the typical disease—is followed by the prompt deposition of the virus in the spleen and bone-marrow, but not in the kidneys. Galloway (Report 1931) has recorded a few experiments in which the infectivity of various organs was determined 24–48 hours after the injection of foot-

and-mouth virus into the pad of the guinea-pig's foot. The concentration of virus was highest in the blood, but the spleen, liver and lungs contained virus in detectable amount, while the mesenteric glands, ovary, testis and muscles did not. In dog distemper (Laidlaw and Dunkin 1928*a*, *b*), organs rich in reticulo-endothelial cells, such as the spleen, show a high virus content.

A review of the available data appears to justify the following conclusions. Where the virus concerned has an affinity for some special tissue in which it causes its characteristic lesions, it will, naturally enough, be found in the greatest concentration in those lesions or in their immediate neighbourhood. Thus we find vaccinia virus in the highest concentration in the lymph of the pustules, the virus of foot-and-mouth disease in the fluid of the vesicles, the virus of rabies, or of poliomyelitis, or of Borna disease, in the central nervous system, and so on; but even in these diseases the other tissues in which the virus is most frequently present, if we except the blood, are those concerned in the normal clearing mechanism—the spleen, liver, lungs, bone-marrow or adrenals. In generalized infections, in which localization in the skin or nervous system is not a feature of the disease, the reticulo-endothelial tissues frequently show the highest virus content.

The blood we must consider in rather more detail. That the virus may often be demonstrated in blood withdrawn during the febrile stage of illness, or even at other times, we have already seen; but many of the results recorded, particularly in the later stages of experimental infections, have been curiously irregular. It would appear that these irregularities have been due in part to the fact that the virus is often present in the cellular elements—particularly in the leucocytes—rather than in the plasma, and that viricidal antibodies may appear in the plasma, while virus is present in the cells.

As long ago as 1899 Kolle showed that the infective agent in the citrated blood of an animal suffering from cattle plague was readily removed by centrifugation. The supernatant plasma was non-infective; the deposit, containing the blood cells, infective to a high degree. Todd and White (1914) studied this phenomenon in greater detail and showed that the virus was mainly associated with the leucocytes, and Schein (1917) and Daubney (1928) recorded similar findings. Russ (1906) (see also Landsteiner and Russ 1906) found that the virus of fowl plague was present in greater amount in the cellular constituents of the blood than in the plasma, and Todd (1928*a*) has shown that the concentration is highest in the leucocytic layer, though Doerr and Gold (1932) record experiments which they interpret as indicating an adsorption of the virus by the red cells.

Smith (1929) has studied this problem in some detail in experimental vaccinia in the rabbit. Comparing the infectivity of the whole blood, the plasma, and the washed deposit of cells during the early days of a generalized infection he found that the washed cells were most infective, and the plasma non-infective. By fractional centrifugation it was possible to show that the red cells played no part in fixing the virus. The results suggested strongly that the leucocytes alone were concerned, but the possible participation of the blood platelets could not be entirely excluded. At a later stage—and this is the immediately significant point—the washed cells might be infective while neither the whole blood nor the plasma produced any lesion in susceptible rabbits. After intradermal inoculation of virus the washed cells might be found infective in animals whose whole blood gave consistently negative results.

In further experiments it was found that specific antibodies made their appearance in the plasma of infected rabbits as early as the 3rd day after infection and thereafter rapidly increased in concentration. There was, therefore, a period during which the leucocytes contained active virus while the plasma contained protective antibodies. Infectivity tests carried out with the whole blood during this period gave irregular and

often negative results, depending on the balance between virus and antibody. But if washed cells, freed from antibody, were used for the test inoculation, virus could regularly be demonstrated in the blood up to the 8th day after infection. Berry and Kitchen (see Russell 1932) in a case of yellow fever noted the simultaneous presence of virus and protective antibodies in the blood on the 4th and 5th days of disease. Such observations indicate clearly that caution should be exercised in assuming that any tissue extract or body fluid is necessarily free from living virus because it is non-infective.

The data that enable us to compare the fate of a virus when injected into an immunized animal with its fate when injected into a normal animal of the same species are even more scanty. There is the clear and significant fact that the characteristic signs of infection do not occur, or are very greatly modified; but beyond that fact we know relatively little.

In the particular case of foot-and-mouth disease, Bedson, Maitland and Burbury (1927) have shown that three stages—not of course sharply demarcated from each other—can be recognized in the active immunization of the guinea-pig. In the first, the animal resists the intramuscular injection of a dose of virus that uniformly causes a generalized infection in controls, but responds to inoculation into the pad of the foot by developing a local vesicle followed by generalized lesions. In the second the solid resistance to intramuscular injection is associated with a heightened resistance to intradermal injection, so that the local vesicle is not followed by generalization. In the third and final stage the guinea-pig is immune to the inoculation of the virus by any route. There is here a clear suggestion of an improved clearing mechanism that, in the partially immunized animal, is able to protect the sensitive tissues if these have not been directly infected at the time of inoculation. Analogous observations have been made during the period of fading immunity in cattle and pigs (Andrews and others; Report 1931). Recent experiments by Galloway (Report 1931) on the survival of foot-and-mouth virus after inoculation into the tissues of immunized animals are in general accord with this view. Virus injected into the mucous membrane of the tongue of immunized rabbits could not be recovered from the blood at any time from the 2nd to the 36th hour after inoculation. Similarly, virus inoculated into the tongue or pads of immunized guinea-pigs could never be recovered from the blood. It could be recovered from the local lesions from 30 minutes to 24 hours after injection, but not after 36 hours.

Of particular interest is an isolated experiment recorded by Smith (1929). Vaccinia virus was injected intravenously into an immunized rabbit, and the washed blood cells were tested for infectivity after 30 minutes, 1, 2, 4, 6 and 8 hours. The cells were infective up to and including the 4th hour, after which they were non-infective. This rapid disappearance of virus from the blood of an immunized animal contrasts sharply with the course of events in a normal rabbit.

The relative parts played by cells and by humoral antibodies in immunity to virus diseases form a subject on which opinion is still sharply divided. It must be understood that, when we use the term "cells" in relation to viruses, we are generally referring not to the reticulo-endothelial cells of the body, which are so active in antibacterial immunity, but to the particular cells invaded by the virus. The reticulo-endothelial system, as we have just seen, undoubtedly acts in conjunction with neutralizing antibodies to dispose of certain types of virus; but when we speak of cellular immunity in virus diseases we have in mind an entirely different mechanism, namely the resistance of normally susceptible cells to invasion by active virus particles. We know that neutralizing antibodies, if mixed with virus before inoculation, or if injected into an animal before the virus, are capable under suitable conditions of preventing infection. Not all virus diseases

can be protected against in this way. In some diseases, for example, such as psittacosis and lymphocytic choriomeningitis, potent neutralizing antisera are difficult to prepare. In others, such as poliomyelitis, though neutralization of virus *in vitro* is possible, injection of serum into a monkey or a cotton rat before inoculation with the virus has no protective effect. The reason for this is probably twofold. Firstly, the virus gains access to the nervous system from the site of injection without entering the blood stream; and, secondly, the cerebrospinal fluid, which is the only fluid in contact with the nerve cells of the brain and cord, does not contain the blood globulins, since the choroid plexus is impermeable to the large molecules of these substances (Perdrau 1938). Moreover, as has already been pointed out, even in virus infections in which a strong neutralizing antibody can be obtained and in which the virus is not essentially neurotropic, the available evidence suggests that inactivation of the virus can occur only extracellularly. Once the virus has penetrated the cell, it seems to be adequately shielded from the antibody. If, as in the prophylaxis of measles, the antibody reaches the majority of the susceptible cells before the virus, the virus may be neutralized either on the surface of the cell or in its immediate vicinity; but if the serum is given too late, after the virus has penetrated the cells, it is almost or completely without effect.

The protective effect of the cell on viruses was studied by Perdrau and Todd (1936). They found that in suspensions free from living animal cells the viruses of fowl plague, herpes, vaccinia, and louping-ill were easily destroyed by photodynamic action (see Chapter 5). But in freshly prepared suspensions of living cells from infected animals they proved very much more resistant. This was not due simply to the presence of the cells, since it was shown that the increased resistance of the viruses did not become apparent for some time after the inoculation of the virus into the animal. Fowl plague virus, for example, inoculated intracerebrally into a fowl, remained susceptible for an hour; vaccinia virus inoculated intratesticularly into a rabbit remained susceptible for about 10 hours; and herpes virus inoculated by the same route remained susceptible for 18 hours. The latent period elapsing before the virus became resistant was interpreted as a measure of the time required by the virus to penetrate the cell and thus obtain protection from the lethal action of the dye. It may be noted that protection was very difficult to establish when the virus was mixed with cell suspensions *in vitro*, unless growing tissues, such as those of the chick embryo, were used. This suggests that penetration of the virus occurs most readily in cells which are either growing or actually dividing.

We see, therefore, that in some diseases in which a second attack is known to be very uncommon neutralizing antibodies appear to play little or no part in the development or maintenance of immunity. Are we justified in assuming that in these instances immunity is purely cellular? This is a difficult question to answer. As Bedson (1937) points out, the mere inability to demonstrate circulating antibody is not sufficient reason for postulating a tissue immunity. It has been abundantly demonstrated that in many virus diseases it is possible to protect a susceptible animal with a dose of serum so small as to render detection of antibody in the circulation highly problematical. It is legitimate to assume, therefore, that, even in the absence of demonstrable circulating antibody, a true humoral immunity may exist due to the inactivating effect on the virus of neutralizing antibody distributed around or on the surface of the susceptible cells. Nor are we justified in concluding that humoral immunity may be ruled out, because no quantitative relationship appears to exist between the amount of circulating antibody and the degree of immunity.

Webster (1938), for example, found that susceptible mice, inoculated subcutaneously or intraperitoneally with 15,000 intracerebral lethal doses of St. Louis encephalitis virus, developed an immunity in 4–7 days to the intracerebral or intranasal inoculation of 1,000 to 1,000,000 lethal doses of virus. The immunity lasted for 4–6 weeks, then declined gradually till it disappeared after 8–12 weeks. While immunity was at a maximum, circulating antibodies were not detectable, though they appeared later when immunity was declining. Webster therefore regards this as an instance of tissue immunity in the absence of humoral immunity. Though this is perhaps the obvious interpretation, it is difficult to accept it without reserve, since we know too little about the distribution of the antibody in the tissues. It may be, as Bedson (1937) suggests in respect of poliomyelitis, that the antibody which appears in the circulation is a response to virus in tissues other than the central nervous system and bears little relation to that in the nervous tissue itself.

Nevertheless, in diseases due to the more strictly neurotropic viruses, of which rabies and poliomyelitis may be quoted as examples, there is so far little or no evidence to suggest that immunity is in any way dependent on the action of humoral antibodies. One attack of the disease appears to confer an almost, though not quite, absolute protection against a second attack, but the mechanism by which this end is achieved is open to doubt. One interesting observation that may throw light on it is the so-called *interference phenomenon*, described by Hoskins (1935) and studied further by Findlay and MacCallum (1937).* It was found that monkeys inoculated with a pantropic strain of yellow fever virus died; but that if neurotropic virus, which is not normally fatal, was injected at the same time, the animals survived. Since the precocious development of antibodies seemed to be ruled out, the explanation offered is that the neurotropic variant occupied a proportion of the cells that would normally have been invaded by the pantropic virus and so prevented them from being infected by this fatal form of the virus. Immunity of this type, in which a cell already occupied by a virus, is resistant to infection by other particles of the same or nearly related virus, may perhaps be referred to as *pre-emptive immunity*—a term suggested by Dr. C. H. Andrewes. We know that such an immunity occurs in plants, in which humoral antibodies have never yet been demonstrated; and there is increasing evidence that it plays a part in the defence of the host against some of the animal virus infections. If this conception is ultimately proved to be correct, then the existence of a true cellular immunity will have been established. At present we must regard it as no more than a plausible guess.

It may be noted that an apparently similar phenomenon has been observed by Delbrück and Luria (1942) in a study of two strains of bacteriophage, α and γ, active against *Bact. coli*. It was found that when sensitive bacteria were exposed simultaneously to the action of both bacteriophages, the γ-phage completely suppressed the growth of the α-phage. It is suggested that, for the multiplication of bacteriophage within the cell a key-enzyme activating the necessary substrate is required, and that for some reason one strain of bacteriophage is more successful than another in competing for this enzyme. It was further shown by Luria and Delbrück (1942) that the interfering action of the γ-phage, though destroyed by heat, was resistant to mild doses of ultra-violet rays sufficient to prevent active growth of the phage itself. The irradiated phage did not lyse the bacteria to which it became attached, but it did prevent their multiplication. These observations are of great interest, and point the way towards the possible use of ultra-violet irradiated vaccines in the prevention of virus diseases.

* For further references, see Mudd, S. (1944) *J. Amer. med. Ass.*, **126**, 632.

The Nature and Reactions of the Antiviral Antibodies.

In discussing the rôle of the serum antibodies in antibacterial immunity, we were able to draw on our extensive knowledge of the ways in which antibodies and bacteria react *in vitro*. Our knowledge of the *in vitro* reactions of filtrable viruses and their specific antisera is as yet rudimentary; but it will help us in the present discussion if we summarize the scanty evidence available, comparing it with that set out in Chapter 7.

In the particular case of vaccinia virus, it has been shown that the serum of an immunized rabbit reacts *in vitro* with virus-containing material from the pocks of variola or vaccinia in man, giving complement fixation and, under suitable conditions, visible precipitation (Gordon 1925, Burgess, Craigie and Tulloch 1929, Bedson and Bland 1929, Craigie and Tulloch 1931, Havens and Mayfield 1931). These reactions are specific—suspensions of material from the lesions of chickenpox do not react with anti-vaccinial sera—and they confirm the antigenic similarity or identity of the viruses of vaccinia and variola. It will be noted that the antigenic material employed is extremely crude. Vaccine lymph contains substances derived from the tissues of the calf and a number of non-pathogenic contaminating bacteria as well as the vaccinia virus itself, and antigenic substances of similar kinds are present in material derived from human skin lesions.

It has been suggested that the reactions referred to above may be due to foreign contaminating bacterial antigens rather than to antigens derived from the virus; but this criticism has been adequately met.

Bedson and Bland (1929) showed that antivaccinial sera did not fix complement in the presence of bacteria derived from calf-lymph, nor did antisera prepared against those bacteria fix complement in the presence of the lymph (see also Havens and Mayfield 1931). Craigie and Tulloch (1931) record the failure of antisera prepared against the secondary bacterial infectors, found in vaccinia and variola crusts, to give visible flocculation with crust extracts. They note, moreover, that antisera prepared by injecting rabbits with extracts of the bacteriologically sterile organs of an animal suffering from generalized experimental vaccinia give good flocculation with crust extracts, and that antisera prepared against dermal vaccine lymph give good flocculation with extracts of virus-containing organs. Similar observations have been recorded by Thompson, Hazen and Buchbinder (1932). The conclusion that these reactions depend on antigens present in or derived from the virus itself is confirmed by the observation of Gilmore (1931) that antivaccinial sera prepared in the rabbit, or human serum derived from cases of variola minor, give specific complement fixation in the presence of vaccinia virus which has been propagated through 17 to 24 successive subcultures in an appropriate tissue medium.

Observations by Ledingham (1931) and by other workers have an important bearing on this problem. The tissues of animals infected with vaccinia may be shown by appropriate staining methods to contain minute granules, the Paschen bodies, which almost certainly represent the actual virus—such bodies may be demonstrated in this and other virus diseases as optically resolved images by the beautiful methods devised by Barnard (1919, 1926, 1932, Barnard and Elford 1931–32). By differential centrifugation Ledingham has been able to prepare suspensions of these elementary bodies almost entirely freed from extraneous material, and finds that, in this state, they are specifically agglutinated by an antivaccinial serum.

Craigie (1932) has recorded experiments which indicate that the flocculation that occurs in a mixture of vaccinia virus and antivaccinial serum is not wholly, or even mainly, an agglutination reaction; but is due in large part to the interaction of a soluble specific

substance derived from the virus with a corresponding antibody in the serum. In the precipitate so formed the virus particles are entangled. The virus particles separated from the soluble substance are agglutinable by the serum, and absorption experiments suggest that the precipitation and agglutination reactions are dependent upon the same antigenic components. Further observations by Craigie, Shedlovsky, Smadel and others (see Chapter 41) have now shown the existence of a soluble, so-called LS, antigen, containing heat-labile and heat-stable components, which reacts with specific antibody in complement-fixation and precipitation tests, but which apparently plays no part in the reaction with neutralizing antibody.

Smith (1932) has recorded experiments that indicate the close similarity between the filtrable substance described by Craigie and the bacterial haptens described in Chapter 7. Rabbits were injected intratesticularly with vaccinia virus, and autolysates were prepared from the infected testicular tissue. From these autolysates a soluble specific substance was obtained, which would withstand boiling, but not autoclaving. It gave a positive biuret test, positive reactions for arginine and tyrosine, negative reactions for tryptophan and cystine, and a positive Molisch test for carbohydrate. It therefore contains both protein and carbohydrate radicles. It shares with the bacterial haptens the capacity to give specific precipitation *in vitro*, and the incapacity to stimulate antibody production *in vivo*.

Douglas and Smith (1930) have studied the action of whole blood and of serum-leucocyte mixtures on vaccinia virus. They find that leucocytes take up the virus *in vitro*; that immune is significantly more viricidal than normal blood; and that normal cells in the presence of immune plasma appear to be more viricidal than immune cells in the presence of normal plasma.

It happens that the greater part of the available data with regard to the *in vitro* antigen-antibody reactions of the filtrable viruses refers to the particular case of vaccinia; but we shall probably not err in regarding this as an illustrative example of the behaviour of viruses in general. All the evidence that we have points in this direction.

Thus Bedson and Bland (1929) have demonstrated specific complement fixation with herpes virus and an antiherpes serum, Ciuca (1929) with foot-and-mouth virus and the corresponding antiserum, Frobisher (1929) with yellow-fever virus and the sera of convalescent men and monkeys, Laidlaw and Dunkin (1931) with the virus of dog distemper, and Broom and Findlay (1932) with the virus of Rift Valley fever and the sera of men, monkeys, sheep, rats and mice that have recovered from natural or experimental infection. Ledingham (1931) has demonstrated the agglutination of suspensions of the Borrel bodies of fowl-pox by the sera of fowls that have recovered from that infection, and Bedson (1932) has demonstrated agglutination and complement-fixation reactions between the elementary bodies of psittacosis virus and a specific antiserum.

It would seem, then, safe to conclude that naturally infected men and experimentally infected animals produce specific antibodies to a filtrable virus, which react with that virus *in vitro* in the same specific way as an antibacterial serum reacts with the homologous bacterium, or with its products, though there is reason to believe that the antigen responsible for the *in vitro* agglutination, precipitation and complement-fixation tests may be different from that which combines with neutralizing antibody.

Certain observations on the neutralization of a filtrable virus by an antiviral serum—as studied by inoculation tests in susceptible animals—have raised doubts as to whether the antigen-antibody reaction involved is entirely similar to that which occurs between a bacterium and the corresponding sensitizing antibody.

Andrewes (1928*b*) found that mixtures of vaccinia virus with antivaccinial serum might fail to give rise to lesions when injected intradermally, indicating effective neutralization, though they proved infective when injected intratesticularly, intracerebrally or intravenously. He found also that virus could be recovered from a neutral serum-virus mixture for periods as long as 24 hours by simple dilution, and that the specific neutralizing

antibodies could be recovered from the mixture by filtration through a candle that held back the virus. Todd (1928c) found that mixtures of fowl-plague virus and anti-fowl-plague serum, which were just neutral when undiluted, regained their infectivity on tenfold dilution with saline. This *dilution phenomenon* was observable after 4 hours' contact between virus and serum at 37° C., but not after 24 hours' contact at 28° C. The effect of dilution at the earlier period was also evidenced by the fact that a mixture that was inactive when injected intramuscularly was infective when injected intravenously. Bedson (1928) records similar experiments with herpes virus and an antiherpes serum, and concludes that there is a slow union between the virus and the viricidal antibodies *in vitro*, the effect of dilution being limited to the period during which this union is incomplete. In later experiments (Bedson 1929) he demonstrated that collodion particles would adsorb the specific antibodies from an antiherpes serum, and that particles so treated would adsorb herpes virus more readily than untreated particles, or than particles treated with normal serum. From this he concluded that the antigen must have united with the antibody. Andrewes (1930) re-examining the reaction between vaccinia virus and an antivaccinial serum in the light of these results found that it became less and less easy to recover active virus from serum-virus mixtures as the period of contact was prolonged, but that the time required for complete irreversible inactivation of the virus was of the order of 24 to 48 hours at 37° C., or 4 to 8 days at room temperature. Similar observations have been made by Shaffer and Enders (1939) and Burnet and Lush (1939*b*) on the herpes virus. The observation of Smith (1930) that it is possible by the use of optimal amounts of absorbing material to absorb separately the antibodies acting on herpes and vaccinia virus from a mixture of antiherpes and antivaccinial serum indicates quite clearly that, under suitable conditions, an effective antigen-antibody union occurs; though Sabin (1935) records experiments from which he draws quite different conclusions.

It would seem probable that antibodies acting on the filtrable viruses form with them a union which is at first of a loose kind, easily dissociable on simple dilution, and which attains stability and firmness only after the lapse of a considerable period of time, probably as the result of some secondary change in the antigen-antibody complex. Closely analogous relations have been shown to hold between certain bacterial antigens and their antisera.

On the other hand, differences from these antibodies have been noted.

For example, in the neutralization of bacteriophage by antiserum, Andrewes and Elford (1933) found that within limits the percentage reduction in the number of active particles was independent of the concentration of bacteriophage in the mixture. Keogh (1936), working with chorio-allantoic membrane cultures, has recorded similar findings in vaccinia. Thus, in one experiment, a 1 : 10 dilution of immune serum, when mixed in equal quantity with a virus suspension, produced a 99·3–99·5 per cent. reduction in the expected number of pocks, irrespective of whether the virus suspension was diluted 1 : 30, 1 : 90, 1 : 270, or 1 : 810. This *percentage law* is supposed to result from the circumstance that within a certain range of dilutions the virus particles are so widely separated that they do not compete with each other for the antibody molecules. A further similarity to phage-antiphage serum reactions was observed in the percentage neutralization of vaccinia virus. Neutralization in constant proportions did not occur. For instance, with one serum studied the pure serum inactivated 99·6 per cent. of virus, a 1 : 10 dilution 99·4 per cent., a 1 : 100 dilution 97 per cent., and a 1 : 1,000 dilution 80 per cent. (See also Chapter 11.)

How neutralizing antibodies act is still subject to discussion.

From the work of Salaman (1937) it seems clear that the neutralizing antibody to vaccinia becomes fixed to the virus particles themselves. Absorption with elementary bodies, but not with the soluble specific substance that takes part in the complement-fixation and precipitation tests, removed the virus-neutralizing bodies from a serum.

The observations recorded above suggest that the neutralizing antibody does not lead directly to the death of the virus; and the finding of virus and antibody together in the circulating blood or in growing tissue cultures in vaccinia (Smith 1929), ectromelia (Downie and McGaughey 1935), and psittacosis (Bedson 1937) is additional evidence in favour of this view. The experiments of Andrewes (1929*a*, *b*) on Virus III and of Rivers, Haagen and Muckenfuss (1929*a*, *b*) on vaccinia and herpes show that in tissue cultures the formation of characteristic inclusion bodies can be prevented by the addition of immune serum to the cultures before the virus. This may be interpreted as meaning that the antibody interferes with the penetration of the cell by the virus, or that it so alters the virus as to prevent its multiplication when it reaches the interior of the cell. Whichever explanation is correct, it is known that the inactivating effect of antibody is increased by the presence of leucocytes, suggesting that, as with bacteria, one of the main functions of neutralizing substances in the serum is to prepare the parasite for phagocytosis and subsequent intracellular destruction.

As is the case with the antibodies acting on bacteria and their products, the antibodies in antiviral sera appear to be confined to the globulin fractions.

Hartley (1914) concluded from the results obtained in the fractional precipitation of anti-cattle-plague serum that the antibodies were contained in the euglobulin. Maitland and Burbury (1927) found that the antibodies in the serum of guinea-pigs immunized against foot-and-mouth virus were associated with the serum globulin. Weyer, Park and Banzhaf (1929) concluded that the antibody of poliomyelitis antiserum was contained in the pseudoglobulin, while Morgan and Fairbrother (1930) found the euglobulin the most potent. Henseval (1919) found the antibodies of a vaccinial antiserum to be distributed throughout the serum proteins, but describes the euglobulin fraction as especially potent. Ledingham, Morgan and Petrie (1931) found antibody in both the euglobulin and pseudoglobulin fractions of an antivaccinial horse serum; the highest concentration was in the euglobulin, but the greatest absolute amount was in the large pseudoglobulin fraction. Laidlaw and Dunkin (1931) found the antibody of distemper antiserum to be confined to the globulin fraction; further study showed that the antibody was associated with a globulin fraction that was insoluble in water, but soluble in weak acids or alkalies and in water saturated with CO_2, thus behaving like the pneumococcal antibody described by Felton (1925, 1926, 1928).

The Antigenic Structure of the Viruses.

The antigenic structure of the viruses has already been discussed in Chapter 41, and we need do no more here than draw attention to the effect of antigenic variation on the degree of resulting immunity.

Just as there are different serological types of pneumococci, each type acting specifically so far as its more important immunity reactions are concerned, so different types of certain viruses are known which seem to behave in an analogous manner. At least three types of foot-and-mouth virus have been recognized, each of which produces the same clinical syndrome in susceptible animals but gives rise to immunity only against itself. Several immunologically distinct types of the poliomyelitis virus have been demonstrated, which are so sharply defined that the serum of monkeys convalescent from one type will not protect against another, and convalescent monkeys immune to re-inoculation with the homologous type can be re-infected with a heterologous type. Other viruses in which antigenic variants occur are those of vesicular stomatitis, African horse sickness, influenza, and the encephalitis group of viruses.

It is clear that the occurrence of multiple serological types should make us very cautious in accepting statements on the effectiveness of the immunity that follows a single attack of a particular virus disease. One attack of foot-and-mouth disease

does not always protect against a second, because the causal viruses in foot-and-mouth disease are not immunologically identical. The occasional second attacks of poliomyelitis that have been recorded (for references see Flexner 1937, Howe and Bodian 1941) may well have been due to infection with a type of virus different from that responsible for the first attack. In influenza the transitory nature of the immunity is probably due to a different cause, since persons convalescent from infection with one type may be re-infected with the same type a few months later (Francis *et al.* 1944); though this does not make it less desirable to pay attention to the infecting type in the vaccine prophylaxis of any given outbreak of the disease.

Allergy in Virus Diseases.

The characteristic response to reinoculation with vaccinia virus affords one of the classical examples of an allergic reaction. The typical sequence of events after a successful primary inoculation commences with an incubation period lasting about 3 days, followed by the appearance of papules at the sites of inoculation on about the 4th day. These develop into compound vesicles during the next 5 days, become definitely pustular about the 10th day, and heal by the well-known scabbing process between the 14th and 21st days. If a person who has been successfully vaccinated is again injected with calf lymph during the period of waning immunity, but before he has again become fully susceptible, the most noticeable feature in the local reaction is an acceleration of all stages. The papules appear earlier, sometimes within 24 hours; and vesiculation and pustulation, when they occur, are in evidence at a far earlier period than after primary vaccination. Very frequently, however, the reaction ceases at the papular stage, and this may be so transitory as to be missed unless daily examinations are made. In any case, the induration round the papules or vesicles is usually far less marked after a secondary than after a primary vaccination, and the constitutional symptoms are slighter. We have, in fact, the typical allergic combination of accelerated response with localization of infection. It would appear (Andervont and Rosenau 1930) that vaccinia virus killed by heating to high temperatures is capable of producing the characteristic immediate reaction with papule formation in persons previously vaccinated; while it has no effect in unvaccinated persons, nor does it produce an active immunity. There are, of course, many analogous observations in the case of bacterial allergy and hypersensitiveness.

There is no reason to believe that other virus infections differ from vaccinia in their ability to induce the allergic state, though this aspect of antiviral immunity has not yet been studied in any detail. A few observations are on record. Thus, Andrewes (1928*a*) records an allergic reaction in rabbits infected with Virus III. Animals that had been solidly immunized showed a relatively small local lesion when reinoculated in the testes, but no nuclear inclusions were present in these lesions. Partially immunized rabbits developed more pronounced testicular lesions, as judged histologically, than did either normal or solidly immunized animals. These lesions, however, unlike those produced in normal rabbits, were usually free from nuclear inclusion bodies.

Other Factors in Virus Infections.

Our knowledge of the interplay of factors other than those we have referred to above is very scanty; but so far as it goes it suggests no fundamental differences between the mechanisms involved in antiviral and antibacterial immunity.

We have seen that the reticulo-endothelial cells are intimately concerned in the pathogenesis of the virus infections, and it seems altogether probable that they play an important

rôle in the production of antiviral antibodies. The time relations of the antibody response to virus infections would seem to be of the same general type as those that occur in bacterial infections, or in active immunization against a bacterial antigen (see Ciuca 1929, Ledingham 1932).

It will be remembered (Chapter 44) that Duran-Reynals (1929) described the enhancement by testicular extract of the skin lesions produced by vaccinia virus in the rabbit—a phenomenon which McClean (1930) showed to be due to a "spreading factor," later identified as hyaluronidase. There is, however, no evidence that the viruses resemble some of the bacteria in producing this substance; so that as far as natural infections are concerned the importance of hyaluronidase would appear to be limited to the theoretical possibility of a mixed infection by a virus and a hyaluronidase-producing bacterium.

A series of observations by M. Maitland (see Report 1928) on the factors determining the localization of the virus in experimental foot-and-mouth disease in the guinea-pig are of considerable interest. Seeking an explanation of the limitation of the vesicles to the mouth and the hairless part of the feet, she transferred strips of hairy skin to the soles of guinea-pigs' feet and found that vesicles developed in the hairy skin so transferred, whether the virus was injected directly into it or introduced elsewhere in the body. She also showed that, when virus was inoculated intramuscularly, vesicles failed to appear on the sole of one hind foot, if that foot was protected by cotton-wool and a bandage. On the unprotected feet vesicles appeared as usual. Again, when the bottom of the cage was made of wire mesh, the pressure on the toes and anterior part of the feet resulted in the appearance of vesicles on these parts, though they are not the usual sites of vesicle formation.

It may perhaps be noted that transmission of virus diseases from the mother to the fœtus or to the new-born child is believed to be very uncommon. According to Levine, Bullowa and Scheinblum (1943), who have summarized the evidence, a few cases of infection *in utero* with varicella, measles, and smallpox have been recorded. Goodpasture (1942) has shown that the placental membrane is impermeable to a number of viruses. On the other hand, specific antibodies are known to reach the human fœtus. This was first demonstrated by Fildes (1915) for syphilitic immune bodies, and has subsequently been proved to hold true for virus diseases such as measles, chicken-pox, poliomyelitis, and influenza (for references, see Levine *et al.* 1943).

Useful reviews, which have been freely drawn on in the writing of this chapter, have been published by Bedson (1937), Burnet, Keogh and Lush (1937), Andrewes (1939), and Rivers (1943).

SUMMARY

It is perhaps a fair summary of the evidence presented in this chapter to suggest that it is compatible with the view that there is no essential difference between the mechanisms involved in antiviral and antibacterial immunity. In so far as immunity to virus infections is more effective than immunity to bacterial invasion, it seems possible that the difference is due rather to the greater limitations imposed on the virus by its habit of intracellular parasitism than to any special reaction on the part of the host.

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CHAPTER 56

HERD INFECTION AND HERD IMMUNITY

In the preceding chapters we have taken as our host unit the individual man or animal. It is clearly possible to work with a different unit—the herd. If we wish to study the epidemic spread of infection, and the factors that favour or prevent it, then it is with this larger unit that we must deal.

The herd, like each of its members, has a characteristic structure; and this structure, from our present point of view, includes not only the hosts belonging to the herd species, and their spatial relationships to one another, but the presence and distribution of alternative animal hosts and possible insect vectors of infection, as well as all those environmental factors that favour or inhibit the spread of infection from host to host. This herd structure, apart altogether from the susceptibility or resistance of the individual hosts, may play a decisive part in the immunity of the herd as such. A herd may be immune to a particular disease—in the logical sense that it will resist the introduction of infection from without—although each of its members is fully susceptible, and would fall an easy victim if he strayed to a herd with a structure that allowed an endemic prevalence of the disease in question. In this sense the English herd is immune to plague; because the association of man, the rat and the flea is not now of a kind to allow spread along natural routes. It is probably immune to cholera, as the result of an adequate system of water purification. It is *not*—nor does it seem likely to become—immune to any of those diseases that are spread by droplet infection. It would take us altogether beyond our present scope to consider the known or problematical effects on herd resistance of such changes in environmental conditions; but we may at least note that many of the most striking successes of preventive medicine have been attained by altering herd structure without inducing any increased resistance in its individual members. By attacking insect vectors of infection, such as the mosquito, by preventing the frequent passage of bacteria from one person's intestine to another person's mouth by way of water and food, and by a general improvement in environmental conditions, we have succeeded in eliminating, or reducing to negligible proportions, diseases that formerly took a heavy toll of lives, and still take that toll in areas where such measures are not applied.

The type of herd immunity with which we are here concerned is that in which this freedom from the spread of infection has not yet been attained, so that contact with the bacterial parasite is at least an occasional event in the normal experience of the host species. Under such conditions the course of events in any infected herd will be determined mainly by the distribution of the parasite and the distribution of those kinds of specific immunity that we have considered in earlier chapters. Intelligent interference with the course of events is impossible without a clear idea as to what is actually happening.

We may recognize, in theory, at least six categories of hosts among any infected herd. Four of these categories include individuals who are themselves infected, (1) the typical case, (2) the atypical case, (3) the latent infection, and (4) the healthy carrier; while two categories are not themselves infected, or infective, (5) the uninfected immune and (6) the uninfected susceptible. The division between (2), (3) and (4) is formal rather than actual—these conditions shade into each other by imperceptible degrees. It is doubtful whether the division between (3) and (4) is justifiable even for convenience of description, since many, perhaps most, of the class commonly described as healthy carriers are in reality suffering from a symptomless, and often negligible, infection. It may be noted that the individuals falling in categories (3) and (4) are in general, though in a very varying degree, resistant to further infection from without; so that the only fully susceptible hosts at risk are those in class 6.

Fig. 268 may help the student to visualize the kind of distribution, both of infection and of immunity, that may be met with in infected herds under different epidemic conditions. No distinction has been made in the diagram between latent infections and healthy carriers. A few arrows have been introduced to indicate the direction of effective spread—effective in the sense of producing new cases of disease or in converting susceptibles to immunes. As we shall see, an epidemic of an infective disease is usually accompanied by an epidemic of symptomless immunization.

In this figure, A may be taken as an example of an epidemic phase in an endemic-epidemic prevalence, that is, as representing the state of affairs during an outbreak of an infective disease from which the affected herd is never completely free, epidemics of varying severity recurring at more or less frequent intervals. B may be regarded as a later stage of A, or as a small epidemic wave occurring in a herd in which susceptibles are few, while carriers are frequent. C may be taken as representing the state of affairs during a severe epidemic occurring in a herd with little initial immunity. An extreme example of this catastrophic type of prevalence has occasionally been afforded by the introduction of such a disease as measles into an isolated island community that has either never experienced the infection before, or has been free from it for many years. D may be regarded as representing a stage of relative quiescence between two outbreaks of the type depicted in A. It will be noted that the proportion of susceptibles is higher than in A or B, and with such a distribution as this a fresh outbreak of the A type is likely to occur.

As an illustration of the types of distribution depicted in A, B and D, no better example could be selected than that of diphtheria. We are here dealing with a disease that is essentially a toxæmia; and, as we have seen, an effective antitoxic immunity will in this case protect the host against clinically detectable infection In the Schick test we have a method which allows us to divide the members of any herd into susceptibles and immunes. Except in the case of very young children a negative reaction may be taken as an indication that an individual has something over 0·01 A.U. of antitoxin per ml. of circulating blood, and also that he will produce further antitoxin briskly and effectively in response to any entry of toxin into his tissues. We may, if we wish, obtain a more exact picture of the distribution of antitoxin, by collecting samples of blood and titrating them by a modified Römer technique (see Jensen 1933). By swabbing throats and noses, and testing the virulence of any morphologically typical diphtheria bacilli cultivated, we can

determine with some approach to accuracy the distribution of the parasite among the hosts at risk ; and we are thus in a position to give a description of the course

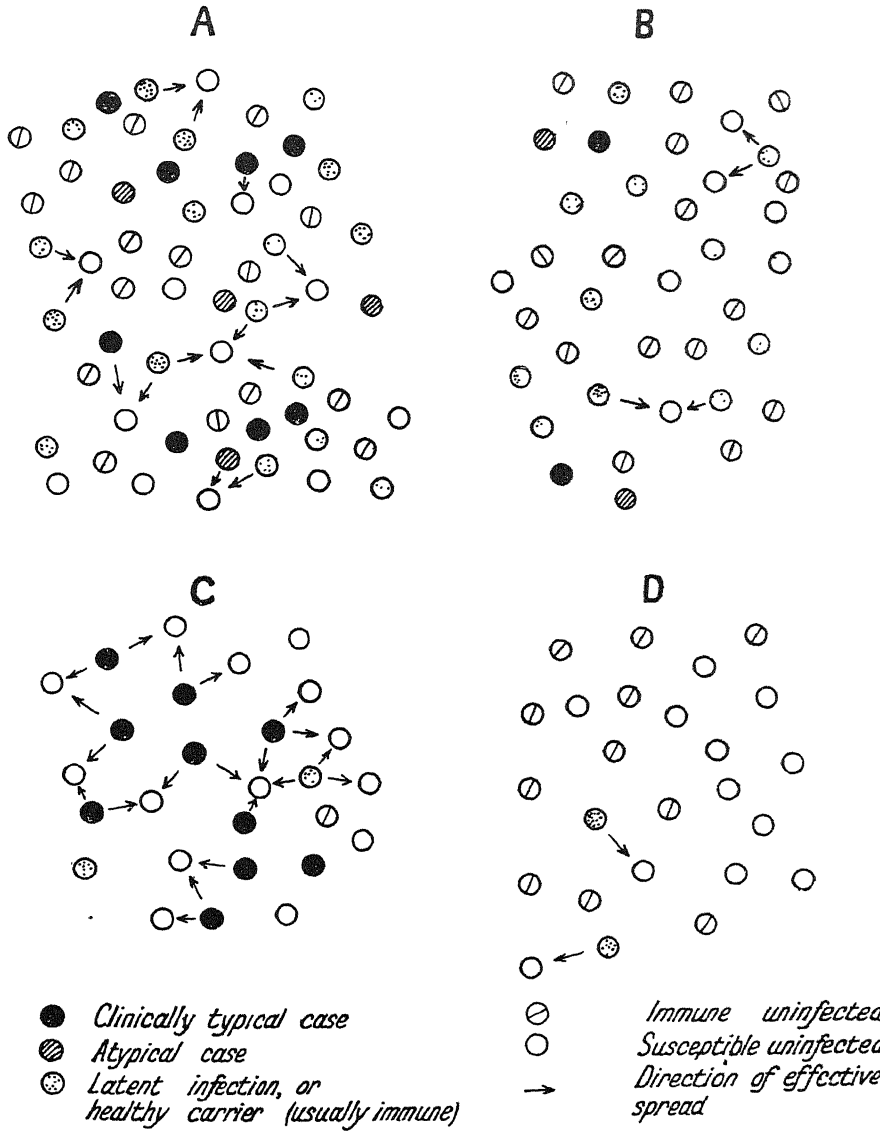


FIG. 268.

of events in any particular community that is vastly more informative than we can at the moment supply for most other diseases.

It has of course long been recognized that the diphtheria bacillus is not confined to those who are suffering from the clinical disease in its typical form. It is

frequently isolated from cases of mild sore throat associated with an epidemic of typical diphtheria, less frequently from healthy contacts, and still less frequently from non-contacts.

Kober (1899) records the isolation of diphtheria bacilli from 70 per cent. of 139 contacts who were themselves suffering from mild sore throat, and from 8 per cent. of 123 contacts who had apparently normal throats. Closeness and continuity of contact, here as in other diseases, have a considerable influence on the carrier rate. The collected figures recorded in the Medical Research Council's monograph on diphtheria (see Monograph 1923) show a 15 per cent. carrier rate of virulent diphtheria bacilli among 610 contacts in barracks or hospital wards, a 7 per cent. carrier rate among 10,883 home contacts, and a 0·6 per cent. carrier rate among the general non-contact population.

As a measure of the risk to which an ordinary urban population is submitted we may take the carrier rate of virulent diphtheria bacilli among children attending the elementary schools in and about London. During the third decade of the century it fluctuated between 2·5 per cent. and 5 per cent. (see Dudley 1923, Forbes 1927).

The events that follow the passage of virulent diphtheria bacilli from the throat of a case, or of a carrier, to the throat of a non-infected person will depend on the immunological condition of the recipient. If he is susceptible (Schick-positive) he will either respond by developing antitoxin and so becoming more resistant, and eventually Schick-negative, or else he will develop clinical diphtheria. Hence Schick-positive carriers of virulent diphtheria bacilli are very rare. They are not non-existent—they cannot be if we accept the view that the transition from the susceptible to the immune class is usually the result of latent infection—but we may take it that a person who is carrying virulent bacilli in sufficient number to be detectable by an ordinary swabbing is (*a*) immune (Schick-negative), or (*b*) undergoing rapid immunization to the Schick-negative level, or (*c*) incubating the disease.

If, then, we take the case of a relatively isolated community, such as a large boys' school, and trace the spread of infection and the development of immunity during an epidemic of diphtheria, we shall observe the following sequence of events.

At the start we may suppose that we have 50 to 70 per cent. Schick-negative immunes and 30 to 50 per cent. Schick-positive susceptibles. Among these boys there will be a certain number of carriers of virulent diphtheria bacilli, say 3 per cent.; these will be immunes.

When these bacilli spread to uninfected boys they may obtain lodgment either in a susceptible or in an immune; and, depending on the dose of bacilli transferred, the exact degree of immunity of the recipient and many other factors of which we have as yet no knowledge, they may bring about any of the following transformations.

Frequently:

(*a*) Schick-positive susceptible → case (mild or severe).
(*b*) Schick-positive susceptible → Schick-negative immune
  (*a*) without detectable carrying,
  (*b*) with detectable carrying.
(*c*) Schick-negative immune → Schick-negative immune carrier.

Very rarely:

(*d*) Schick-positive susceptible → Schick-positive carrier.
(*e*) Schick-negative immune → mild "bacteriological" case.

As an illustrative example of the findings that have actually been recorded in work of this kind, we may cite the illuminating studies of Dudley (1923, 1926, 1932) on diphtheria prevalence during a series of years in the Royal Naval School at Greenwich, a school of some 1,000 boys.

Between January and May 1919—the period at which Dudley's record starts—there were 65 cases of diphtheria. From then onwards until May 1921 there were no cases. Between May and July of that year 20 cases were recorded, and in the September-to-December term the outbreak reached epidemic proportions, with 77 cases. Between January and April 1922, 831 of the boys were Schick-tested. The results obtained are summarized in Table 84.

TABLE 84

SHOWING RELATION BETWEEN SCHICK REACTION AND PREVIOUS EXPERIENCE AMONG BOYS TESTED JANUARY TO APRIL 1922.

Previous Residence in School.	Experienced 1919 Epidemic.	Experienced 1921 Epidemic.	Per cent. Immune (Schick-negative)
None (new boys) . . .	No	No	58
6 months–2 years . . .	No	Yes	85
Over 3 years	Yes	Yes	95

The relation between experience and immunity is obvious. Moreover, the fluctuations in the immunity rate as plotted by terms of entrance were discontinuous. For entrants during the four terms September 1917 to September 1918 inclusive the percentage giving negative reactions in 1922 were 100 per cent., 92 per cent., 100 per cent. and 98 per cent. respectively. For the entrants during the period May 1919 to May 1921 inclusive the rates by terms of entrance were 82 per cent., 90 per cent., 82 per cent., 85 per cent., 83 per cent., 82 per cent. and 86 per cent. as compared with the 58 per cent. in boys joining in January 1922. It would appear that there had been two waves of immunization in the school, coinciding with the waves of clinical infection.

A sample swabbing of the school during the latter part of the 1921 epidemic showed 28 carriers of diphtheria bacilli in addition to the 77 cases. During the period January to July 1922 the carrier rate for virulent diphtheria bacilli was 2·2 per cent., during September to December of the same year it rose to 4·5 per cent., and during this period cases of diphtheria were still occurring. But immunization of susceptibles was proceeding synchronously with the spread of clinically detectable infection. Of 88 boys who had given positive reactions on first testing 24 reacted negatively when retested 3 months later. Similar findings are recorded for later years, with carrier rates of virulent diphtheria bacilli varying from 1·0 to 4·5 per cent., and with recurrent cases of diphtheria that never attained the epidemic frequency of September to December 1921. Thus of 35 Schick-positive entrants in March 1924, 13 had become negative within 4 months; and 2 years' residence in the school had increased the 58 per cent. immunity rate of the new boys entering in January 1922 to 84 per cent. It may be noted that this rapid immunization was confined to the boarders, forming the population of about 1,000 to which all the figures quoted above refer. There were at the school some 120 day boys, who did not share in the continuous exposure to risk of infection experienced by the boarders. Among these the rate of immunization was relatively slow. Over a 4-months' period only one of 24 Schick-positive day boys became Schick-negative.

The increasing ratio of carriers to cases as a herd becomes more and more immune is well illustrated by the case rates and carrier rates in one group of some 160 boys during the winter term of 1921 and the winter term of 1922.

	Case Rate.	Carrier Rate.
1921	7 per cent.	2 per cent.
1922	0·6 „ „	6 „ „

In 1921 during a severe epidemic, following on a period in which the school had been free from diphtheria for 2 years, the spread of virulent bacilli produced 3·5 cases to one carrier; in the winter of 1922 after this severe exposure, and in spite of the entrance of 43 new boys during the post-epidemic period, the spread of infection produced 10 carriers to one case.

In judging the significance of such figures we must remember that the real carrier: case rate over any considerable interval of time is always higher than that given by comparing the recorded case rate with the recorded carrier rate over that period—a point stressed by Dudley (1932). All clinical cases of diphtheria will be recorded. A case rate of 3 per cent. per annum in a particular community means that 3 persons in every hundred of those exposed to risk develop diphtheria during the year in question. But a carrier rate of 7 per cent. does not mean that 7 per cent. become carriers during the year; it means that at any one swabbing of an adequate sample 7 persons in each hundred are, on the average, found to be harbouring diphtheria bacilli. The total number of persons that become carriers during the period in which the three cases of diphtheria occur will be much higher. Thus, over one yearly period, Dudley records an average carrier rate of 6·6 per cent. with small fluctuations above or below this level; but repeated swabbing (7–8 times) of a large sample of boys showed that at least 40 per cent. were harbouring the diphtheria bacillus at one time or another during this period.

The general significance of the picture presented by such studies as these is clear enough; but we must remember that the two categories—immunes and susceptibles—that are divided from one another by simple Schick testing present a very incomplete picture of the graduations in resistance that actually exist in a herd at risk. Glenny (1925) defines five grades of immunity, which are set out in Table 85. These are, of course, not exhaustive; nor are they sharply demarcated from one another (see also Parish and Wright 1938).

TABLE 85

SHOWING VARYING GRADES OF RESISTANCE TO DIPHTHERIA. (After Glenny.)

	Group.				
	1	2	3	4	5
Schick test	−	+	+	+	+
Antitoxin in blood	> 1/30 A.U.	< 1/30 A.U.	0	0	0
Earlier stimuli	Many	Many	Many	Few	None
Response to further stimuli	Rapid	Rapid	Rapid	Slow	Very slow
Description	Immune	Immune	Potentially immune	Sub-immune	Fully susceptible

If we could elaborate our study of a population at risk by estimating at regular intervals the exact amount of antitoxin in each person's circulating blood; and if, dealing with a very large population, we could withdraw an adequate sample of persons at frequent intervals and determine the response of each Schick-positive person to small injections of toxoid, we should be able to build up a far more detailed picture of what was really happening. Adopting Glenny's classification, we should be able to detect the transformation from the fully susceptible to the sub-immune, and thence through the Schick-positive immune to the fully developed Schick-negative immune, as well as transference from the Schick-positive to the Schick-negative class.

Our rare Schick-positive carriers would be found in the immune or potentially immune sub-groups, and would rapidly pass into the Schick-negative class. We should almost certainly find that severe cases tended to arise from the fully susceptible Schick-positives, mild "bacteriological" cases from the sub-immunes or potentially immunes.

A point of great importance is the reaction of the Schick-immunes to the recep-
ɔn of virulent diphtheria bacilli. We know that they very rarely contract clinical
phtheria, and then only in a mild form. We know that many of them become
rriers. Is a point reached in natural immunization at which a person resists
rrier infection as well as infection in the clinical sense ? Can we grade our Schick-
ɪmunes as we grade our Schick-susceptibles, putting at the lower end of the scale
ɹose who have just attained to the Schick level of immunity, and at the upper end
the scale those who are so resistant that virulent diphtheria bacilli are unable
gain lodgment in their throats ? If a human herd is rendered 100 per cent.
ɔhick-negative, either by natural immunization or by prophylactic inoculation,
ill infection with the diphtheria bacillus be eliminated in the bacteriological sense,
ɾ will a freedom from clinical diphtheria be associated with a persistent carrier rate
ot much below that of the population at large, and perhaps with carrier epidemics ?
ɜ complete immunity, of the kind that would render a herd entirely free from in-
ɛction, likely to be attained by immunization of this kind, however far it is pushed ?

These questions have been answered to some extent. In America and Canada,
xtensive immunization of children has resulted in a significant reduction of clinical
liphtheria in the community treated, and in some cases the disappearance of the
lisease (see Russell 1943). The effect of immunization on the carrier rate appears
ɔ vary with the population. In small institutional communities, for example,
mmunization may be followed by a decline of clinical diphtheria and a rise in the
ɜarrier rate. In larger, more open communities, the increase in the number of
Schick-immunes is not accompanied by an increase in the carrier rate (see, for
example, Fitzgerald *et al.* 1938). These issues are fully discussed in Chapter 61,
but we may note here, that in so far as we are dealing with a purely antitoxic
immunity—and this is the case in our artificial Schick-immunes—we should not
expect freedom from the carrier state unless successful lodgment in the throat
depended on a local toxic effect. In so far as our natural Schick-immunes possess
an antibacterial as well as an antitoxic immunity we must remember that the
diphtheria bacillus is separable into many antigenically different types, though
all types produce the same toxin (Durand 1918, 1920, Havens 1920, Smith 1923,
Eagleton and Baxter 1923), so that we should expect any antibacterial immunity
to be type-specific. Moreover, it is very doubtful whether the mechanisms dis-
cussed in Chapter 47 would have any appreciable effect in preventing a localized
tonsillar infection.

Scarlet fever is another disease in which, by the aid of the Dick test and the
examination of throat swabs for hæmolytic streptococci, we can obtain information
of the same general kind as that now available in the case of diphtheria ; but we
have not yet any records comparable in detail and duration with those afforded
by Dudley's work at Greenwich. So far as our fragmentary records go they look
like pieces of a very similar picture, with one essential difference. The production
of the erythrogenic toxin forms a relatively small part of the pathogenic poten-
tialities of hæmolytic streptococci ; and an antitoxic immunity that will suffice
to prevent the occurrence of the clinical syndrome that we label scarlet fever will
not suffice to prevent the spread of tonsillitis, or of other clinically obvious strep-
tococcal infections (see Okell 1932). For effective immunity against all the clinical
manifestations of infection with hæmolytic streptococci we need an antibacterial
as well as an antitoxic immunity ; and this antibacterial immunity will almost
certainly be type-specific.

Turning to diseases in which antitoxic immunity, in the strict sense in which we are using that term, plays no apparent part, we are not in a position to give any adequate description of the course of events in a naturally infected herd. This is because we have no such simple tests as the Schick and Dick reactions by which to separate our immunes from our susceptibles. There are good reasons for believing that by an adequate survey, including a careful study of the distribution of the infecting organism and tests for the presence of specific antibodies in the blood of the hosts at risk, we could add greatly to our knowledge of what is happening during an endemic or epidemic prevalence. But we have not got that knowledge yet.

Some things we do know. We know that in certain diseases, such as cerebrospinal meningitis, the ratio of carriers to cases is very high (see for instance Glover 1918), so that it seems probable that the immunes greatly outnumber the susceptibles. The same general relationship—a widespread carrier epidemic associated with relatively few clinical cases—seems to hold in certain virus diseases, such as poliomyelitis and encephalitis lethargica.

In other diseases, such as typhoid fever, the ratio of carriers to cases is not so high (see Chapter 69). But we know that atypical cases of typhoid fever occur during an epidemic, and that many carriers of typhoid bacilli give no history of ever having suffered from a typhoid-like disease. It is a fairly safe assumption that an endemic or epidemic prevalence of typhoid fever is associated with the occurrence of sub-clinical immunizing infections. We know quite certainly that such a prevalence leaves behind it infected healthy carriers who may be a potent source of further spread.

THE EXPERIMENTAL STUDY OF HERD INFECTION AND IMMUNITY

It is clearly possible, by selecting a convenient host species and a parasite that spreads naturally among them, to submit problems of the kind we have considered above to direct experimental study—initiating an epidemic of a particular disease among our test population, and studying the reactions of new entrants, of old members of the herd, or of migrants from one herd to another, by any available means (see Greenwood *et al.* 1936). We can, under such conditions, observe the effects of any intentional interference we choose, splitting a herd into smaller units, reaggregating these units after any selected interval, varying the rate of immigration of susceptibles, actively immunizing some or all of our animals by vaccines of different kinds administered by different routes, and so on as new problems suggest themselves for attack. In this way we gain enormously in an increased control over many of our unknown variables; but we lose, in so far as we wish to argue from our experimental herds to happenings in the natural world outside our cages, by having to rely on analogies that are certainly incomplete, and may well be misleading. Because our control is still far from complete we must use large numbers of animals and make constant use of statistical methods in trying to assess the meaning of our results. We must, therefore, use a small, easily controlled and relatively inexpensive animal, and the mouse fulfils these requirements. Experiments of this kind have been carried out during a number of years, in England and in America, on mouse typhoid—caused by infection

with *Salm. typhi-murium* or less frequently with *Salm. enteritidis*—, on mouse pasteurellosis, and on a virus disease known as ectromelia. The American workers have also studied the spread of infection among rabbits and fowls housed in laboratories, or maintained as breeding stock by dealers. It will be convenient to consider the data thus obtained in direct relation to the observations on human herds, noting carefully the points at which particular caution must be exercised in applying the argument from analogy and in generalizing from an isolated experience.

Is there, in the spread of infection among mice, the same distribution of typical cases, atypical cases, latent infections and carriers, and the same natural immunization associated with natural infection, that occurs among human herds?

In the particular case of mouse typhoid—and almost certainly in other epidemic infections—it is quite certain that the great majority of the mice at risk are infected by the bacterial parasite at some period or other during a prolonged epidemic prevalence.

In a particular experiment (Topley, Ayrton and Lewis 1924) 5 mice were fed on a culture of *Salm. typhi-murium*. As soon as they were found to be excreting the bacillus in their fæces, 20 normal mice were added, and thereafter 1 normal mouse was added to the cage each day for 115 days. The fæces of every mouse were examined on 6 days a week during the course of the experiment; each mouse that died was examined bacteriologically, and at the termination of the experiment all surviving mice (55 in number) were killed, cultures were taken from their spleens, and their blood was tested for agglutinins. The existence of non-fatal, and apparently mild or trivial, infections was clearly demonstrated. Some mice that excreted *Salm. typhi-murium* on several occasions remained in apparent health during the whole period of their residence in the cage. When these were killed at the end of the experiment some gave negative spleen cultures and showed no agglutinins in their blood, others gave negative spleen cultures but possessed agglutinins, others gave positive spleen cultures with or without agglutinins in the serum. Taking, as criteria of infection, death from the infective disease, excretion of the organism in the fæces, and the isolation of the organism, or the demonstration of agglutinins, in the surviving mice, it was found that of 128 mice which had resided in the cage for periods of 14 to 115 days, 122 had become infected. Of these infected mice 46 were surviving in apparent health on the day when the experiment terminated, but 25 of them (54·3 per cent.) were found to be harbouring *Salm. typhi-murium* in their spleens. The collected figures for eleven different epidemics of mouse typhoid, lasting from 60 to 117 days, show that of 267 surviving mice 190 (71·2 per cent.) were latently infected (Topley 1926).

Similarly (Greenwood *et al.* 1936), the results obtained in experiments with the virus disease, ectromelia, indicated that some 80 per cent. of the mice entering the herd were infected with the virus within three weeks of entry.

The way in which the bacterial parasites are distributed among the hosts at risk is then of the same general kind as obtains in natural epidemics in man, differing mainly in the fact that fewer hosts escape infection. Is there any evidence that the spread of infection results in immunization as well as, or in place of, the production of overt disease? That survivors from an epidemic are, on the average, more resistant than new-comers to an infected herd is certain; for they live longer —usually much longer—when exposed to a subsequent wave of mortality (Topley 1921, Amoss 1922).

This increase in resistance with increasing herd experience under epidemic conditions can be studied in greater detail by constructing life tables for the mice

submitted to risk of infection during a long-continued prevalence of such a disease as mouse typhoid. It is convenient in calculating the expectation of life to limit it to 60 days, regarding any mouse living for longer periods as dying on the 60th day, since the use of the unlimited expectation of life gives undue weight to the relatively few mice that live for much longer periods, and also because we have a normal standard of comparison for the shorter period, but none for the latter.

Employing this value (${}_{60}E_x$) we find in a particular experiment, in which 3 normal mice were added to an infected herd each day from March 6th, 1921, to June 30th, 1923, that the limited expectation of life drops slightly (from 22·49 to 21 days) during the first week of herd life and then rises, at first slowly, but more steeply after the 15th day, until by the 33rd day it reaches the figure of 34·75 days. It fluctuates round this figure up to the 100th day of herd residence; after which the numbers of survivors are too small to give a reliable average. In general then, we may say that a mouse that has survived about 30 days' exposure to risk will live more than half as long again as a new-comer to the cage (Greenwood and Topley 1925, Greenwood *et al.* 1936).

We can ask the same question in another way, using a direct experimental comparison instead of a life table. In a particular experiment (Greenwood, Newbold, Topley and Wilson 1926) in which mouse pasteurellosis was the infection selected for study, two parallel epidemics (A and B) were initiated on November 11th, 1924, and were maintained by the addition of normal mice until December 1925. At regular intervals groups of mice that had survived for 10, 20, 30, 40, 50 days or more in Herd A were transferred to Herd B, and with them were added a numerically equal group of normal mice that had had no previous experience of pasteurellosis. Averaging the results, the figures shown in Table 86 were obtained:

TABLE 86

Length of Life in Herd A.	Expectation of Life in Herd B (${}_{60}E_x$).
Nil (normal entrants to B)	22·37 ± 0·36 days
10 days	21·34 ± 1·10 ,,
20 ,, ...	25·50 ± 1·13 ,,
30 ,, ...	32·55 ± 1·28 ,,
40–45 days	33·08 ± 1·52 ,,
50–60 ,, ...	37·39 ± 1·69 ,,

Here, again, survival through a testing period of 30 days or more added some 50 per cent. to a mouse's expectation of life under severe epidemic conditions.

But clearly we cannot, in the absence of further evidence, assume from such results that the mice have been actively immunized. We are dealing with fatal diseases, and natural selection has certainly been at work. In the mouse typhoid experiment, of every 1,000 mice living on day 0 (their day of entry to the herd) only 256 survived for 30 days. Death had by then removed some 75 per cent. of those at risk; and, if no active immunization had occurred, the surviving 25 per cent. would have a higher average resistance than new-comers to the herd, assuming only that the natural resistance of mice differs *inter se*, and that this difference plays some significant part in determining survival during the first 30 days—an assumption that may be very safely made. Webster and his colleagues (see Webster 1923*a*, *c*, *d*, 1924*a*, *b*, 1926, Pritchett 1925, 1926) consider that this natural, inborn resistance is the dominant factor in determining death or survival during an epidemic. That it plays some part there can be no reasonable doubt, but there are reasons for doubting its dominance, and for believing

that active immunization is exerting an effect similar in kind, if less in degree, to that exerted in the spread of diphtheria in man.

Thus (Greenwood *et al.* 1926, 1928, Newbold 1927), if the method of partial correlation is applied to the data for mouse typhoid and mouse pasteurellosis, and correlation coefficients are calculated for: (A) length of after-life and length of previous exposure, keeping constant the death rate during previous exposure and the death rate during after-life and (B) length of after-life and death rate during previous exposure, keeping constant the length of previous exposure and the death rate during after-life, we get the figures set out in Table 87.

TABLE 87

Coefficient.	Nature of Epidemic.		
	Pasteurellosis. (1)	Pasteurellosis. (2)	Mouse Typhoid.
A	$0{\cdot}194 \pm 0{\cdot}016$	$0{\cdot}150 \pm 0{\cdot}015$	$0{\cdot}307 \pm 0{\cdot}014$
B	$0{\cdot}029 \pm 0{\cdot}017$	$-0{\cdot}018 \pm 0{\cdot}016$	$-0{\cdot}282 \pm 0{\cdot}014$

Coefficient A is positive and significant in all cases, indicating that the longer a mouse has survived within limits under constant conditions as regards death rate, the longer it will continue to survive under continued exposure to infection. Coefficient B is insignificant in the two pasteurellosis experiments, significant but negative in the mouse typhoid experiment, indicating that, with equal lengths of exposure to risk, the survivors from a period of high mortality have no significant advantage over the survivors from a period of lower mortality. This latter finding clearly suggests that the elimination by death of the more susceptible mice is not a very important factor in raising the average resistance of the survivors, while the advantage derived from prolonged exposure at a constant death rate is compatible with the view that active immunization is important. The exact significance of these results is, however, uncertain. It is probable that some at least of the mice emerging from a period of high mortality will already be suffering from an infection which will cause their death in a few days or weeks, apart altogether from any subsequent risks, while it may be that fewer of the mice emerging from a more prolonged exposure to a lower rate of herd mortality are in this particular position.

The probability that active immunization is an important factor is, perhaps, increased by the observation that, in the transfer experiment with pasteurellosis, mice that had lived in herd A only during periods of low death rates—less than one-third the average death rate for the whole epidemic period—still showed a marked advantage over normal mice on transference to herd B. The limited expectation of life of those normal immigrants that entered herd B on the same days as these particular groups of migrants from herd A was $20{\cdot}9 \pm 0{\cdot}49$ days, for those migrants that had spent 50 days in herd A it was $34{\cdot}6 \pm 4{\cdot}10$ days.

It may be noted also that such evidence as is available (Topley, Wilson and Lewis 1925, Greenwood and Topley 1925) indicates that the increased resistance gained by survival in an infected herd, or after experimental infection by feeding or inoculation, is specific in the ordinary bacteriological sense. A mouse that has survived infection with *Salm. typhi-murium* appears to be no more resistant than normal mice to infection with *Pasteurella muriseptica*. This, of course, accords well with epidemiological experience in general, but not with the view that the determining factor in increasing the average resistance of a herd at risk is the selection by the sieve of death of those individuals possessing an innate non-specific resistance. If differences in innate resistance are of major importance it would appear that they must either be specific or must be differences of immunizability rather than of immunity.

Whether or not active immunization is the main factor that determines the increased average resistance of surviving mice, the grade of antibacterial immunity produced can excite little enthusiasm.

As stated above, the expectation of life limited to 60 days was selected in these and subsequent experiments in part because there is an available standard of reference for this period (Greenwood, Topley and Wilson 1931*b*). On October 4th, 1929, 20 normal mice were assembled in a cage of the same type as that used in all epidemic experiments. From then onwards until January 17th, 1930, daily additions of normal mice were made, and the herd was then observed until May 27th, 1930. The 329 mice of this experiment were living under exactly the same conditions as the infected herd, except that no infection was present at any time during the period of observation. Of these 329 mice 56 died. The limited expectation of life, as was to be expected, remained practically constant at all cage ages from day 0 to day 170—the last day for which figures were available. The lowest ${}_{60}E_x$ figure was 56·69 days, the highest 59·27 days. The figure rose slowly from just under 57 days on the day of entry to over 58 days on the 40th day of cage life—presumably as the animosities of first acquaintance were replaced by the toleration of later herd life and fighting grew less frequent. Thereafter it hardly varied.

Taking a figure of 58 as our normal ${}_{60}E_x$, we note that the best that natural immunization against mouse typhoid or mouse pasteurellosis can do—so long as surviving mice are exposed to a continuous risk of heavy infection—is to raise the expectation of life from a little under a half to a little over two-thirds of the normal figure. This is a very different picture from that presented by our study of diphtheria in man—even allowing for the fact that in the latter case we are dealing with a disease of relatively low fatality. Either mice differ in some fundamental way from men; or the conditions in mouse cages differ fundamentally from those in schools and institutions—an obvious possibility; or natural immunization against enteric infection or against pasteurellosis is less efficient than natural immunization against diphtheria or scarlet fever. Perhaps, though by no means certainly, this might be expanded to the conclusion that natural antitoxic immunization is much more effective than natural antibacterial immunization.

There is no known disease of mice that presents any close analogy with such toxæmic human infections as diphtheria and scarlet fever; but the description by Marchal (1930) of a natural virus disease of mice—ectromelia—that gives rise to severe and fatal epidemics under experimental conditions has afforded an opportunity for the study of natural immunization against a virus infection. The results obtained present a striking contrast to mouse typhoid or mouse pasteurellosis (Greenwood *et al.* 1936).

Adopting the same plan of starting an epidemic in a herd of mice and thereafter adding 3 mice a day, two epidemics of this disease have been observed over a period of some 18 months. Applying the ordinary life-table method it was found in one of these epidemics —the results in the other were entirely analogous—that the limited expectation of life (${}_{60}E_x$) on entry to the infected herd was 30·71 days. It fell until the 7th day when it was 25·39 days, this fall obviously depending on the average time taken to contract infection. From then onwards the ${}_{60}E_x$ value rose; but instead of reaching a maximal value in some 30 days at a figure far below the normal expectation of life it continued to rise until, on the 100th day after entry, it had reached the figure of 54·69 days, and thereafter fluctuated slightly above and below this value over a period of some 15 months. In other words, instead of experience and survival in the infected herd resulting, at the best, in an expectation of life of some two-thirds of the normal span, those mice that lived through some 3 months or more of the epidemic prevalence had an expectation of life little inferior to

the normal ${}_{60}E_x$ value of 58 days, though they continued to live in a herd in which the specific infection was causing a high and continuous death rate.

A more prolonged experience of the same epidemic prevalence has shown that waves of exceptionally high mortality occur during which many of the old survivors succumb; but this fact does not abolish the significance of the marked difference in behaviour between a bacterial and a virus infection over epidemic periods that last through a large part of the lifetime of a normal mouse. It seems fairly clear that, even under conditions of severe and continuous exposure to infection, a mouse can develop a relatively effective immunity to ectromelia.

How far may the course of events in an infected herd be modified by the artificial immunization of some or all of the hosts at risk?

We shall discuss in later chapters the evidence with regard to the efficacy of different kinds of prophylactic vaccination in human herds. So far as experimental epidemiology is concerned we can, at the moment, attempt an answer only in mouse typhoid and, with far scantier data, in ectromelia.

It is quite certain that active immunization with a killed culture of *Salm. typhi-murium* will increase the resistance of mice to that organism; and although some of the earlier observations (Loeffler 1906, Wolf 1908, Yoshida 1909, Brückner 1910, Webster 1922, Ornstein 1922, Neufeld 1924, Lange and Yoshioka 1924) suggest that the immunity obtained in this way is relatively poor and inconstant, repeated trials with considerable numbers of mice, and with carefully adjusted doses of vaccine and of living culture, indicate that a quite significant increase in resistance can regularly be obtained (Topley 1929, Topley, Wilson and Lewis 1925).

Thus, in one experiment, 50 mice were immunized by two intraperitoneal injections of 5×10^8 *Salm. typhi-murium* killed by formalin and heat. A week after the second injection these 50 immunized mice, together with 50 normal controls, were injected intraperitoneally with 1,000 living *Salm. typhi-murium*. The results are shown in Table 88 This vaccine had, therefore, produced the following effects: a proportion of the immunized mice showed a partial but ineffective immunity in that they lived on the average far longer than the controls but succumbed to the disease during the period of observation; 24 per cent. showed a more effective resistance by living in apparent health throughout the period of observation, but post-mortem examination showed that they were suffering from a latent infection; 18 per cent. appeared to have been rendered completely immune to this dose of bacteria, in that they remained in apparent health for 28 days and showed no evidence of infection when killed and examined by spleen culture. These results may be taken as representative of a considerable number of similar experiments.

TABLE 88

	No. Inoculated.	No. Dead in 28 Days.	Average Time to Death of Mice that Died.	No. Survivors with Positive Spleen Cultures.
Normal	50	50	5·0 days	—
Vaccinated . . .	50	29	18·3 „	12

We should not perhaps expect an immunity of this order to be very effective as a prophylactic measure in herds submitted to a continuous risk of infection, and actual trial confirms our lack of faith.

An epidemic of mouse typhoid was started on January 1st, 1928, and was continued until September 17th, 1929, by the addition of 3 normal mice a day (Greenwood, Topley and Wilson 1931*a*). Every 7th day 10 additional normal mice, and several groups, each of 10 mice, that had been immunized with different vaccines were added to the cage. The results, as they concern the relative efficacy of different antigenic components, have already been noted on p. 1056. We are concerned here only with the results obtained with the most effective vaccines employed.

The limited expectation of life ($_{60}E_x$) of the normal mice on entry was 26·26 $\pm$ 0·641 days; for the four immunized groups that had received vaccines containing the smooth surface antigen the $_{60}E_x$ figures were 32·10 $\pm$ 0·432, 34·97 $\pm$ 0·845, 35·06 $\pm$ 0·845 and 33·71 $\pm$ 0·853 days respectively. Thus by active immunization with a killed vaccine an increase in average resistance was obtained of the same order as that attained naturally by normal mice after living for some 30–50 days in an infected herd under the joint influence of active immunization and mortuary selection.

After 60 days' residence in the cage the $_{60}E_x$ figures for the vaccinated and unvaccinated groups have, as we should expect, largely levelled up, though the vaccinated mice still show a slight advantage. The figure for the unvaccinated group has risen to 36·47 days, those for the vaccinated groups to 40·17, 37·39, 36·90 and 47·54 days respectively.

Very similar results were obtained in another experiment in which a comparison was made between the effect of administering a killed *Salm. typhi-murium* vaccine by the mouth, or by intraperitoneal inoculation (Greenwood, Topley and Wilson 1931*c*). A larger dose was used for *per os* than for intraperitoneal immunization ($5{,}000 \times 10^6$ as against 500×10^6 bacilli). In each case two doses of vaccine were given with a week's interval between them, and the mice were added to the infected herd 1 week after the second dose of vaccine. The experiment lasted from December 12th, 1929, to June 26th, 1930. The death rate in this epidemic was rather lower than in those referred to above, and the expectation of life of the normal mice on entry was therefore somewhat longer. The $_{60}E_x$ figures on the day of entry were as follows: normal mice 31·18 $\pm$ 1·04 days, mice vaccinated *per os* 35·30 $\pm$ 1·22 days, mice vaccinated intraperitoneally 38·19 $\pm$ 1·17 days.

The immunity to ectromelia induced by vaccinating mice with a formolized virus is far more effective than the immunity to mouse typhoid induced by vaccination with a killed suspension of *Salm. typhi-murium*. Thus, in one series of tests (Greenwood *et al.* 1936), 173 of 180 vaccinated mice survived a dose of ectromelia virus that killed all of 60 controls.

The expectations raised by these results were fulfilled, up to a point, in the single experiment in which the effect of active immunization against the virus disease has been tested under epidemic conditions (Greenwood *et al.* 1936).

For this experiment, a herd was selected in which ectromelia was actively spreading, and causing a high mortality. On 21.10.32, and on every seventh day thereafter for a period of 70 days, 30 immunized and 30 non-immunized mice were added to the herd. From 21.10.32 to 27.3.33 three additional normal mice were added daily. The experiment was terminated on 16.3.34. Taking the 300 immunized mice, the 300 normal controls added with them and the 210 normal mice added daily over the same period, the $_{60}E_x$ values for the day of entry to the herd were: immunized mice 49·1 days, normal controls added with immunized 20·8 days, normals added daily 21·4 days. This shows a very great advantage to the immunized mice, and it was maintained throughout later cage-ages until the combined effects of natural immunization and selection, which are so effective in this disease, had raised the $_{60}E_x$ of the surviving normal entrants to the same high level.

In the final stages of this epidemic there were many deaths among the artificially immunized mice, and among the naturally immunized survivors. Whether these deaths

were in all cases due to ectromelia it is very difficult to say. It will, in any case, be wise to withhold any final opinion as to the efficacy of immunization in affording permanent protection against ectromelia under these very severe conditions of exposure until further data are available.

The evidence so far recorded may conveniently be summarized in tabular form (Table 89). The ${}_{60}E_x$ figures for the surviving mice have been recorded after that period of exposure (30–100 days) when the full advantage appears to have been attained, and the limited expectation of life has reached relative stability. For the uninfected standard herd the figure of 58 days has been taken both for new entrants and for survivors at all periods. The two sets of figures for the vaccinated mice in the second mouse typhoid experiment refer to the least and most effective of the four antigenically similar vaccines employed.

TABLE 89

SHOWING LIMITED EXPECTATION OF LIFE (${}_{60}E_x$), IN DAYS, FOR VARIOUS GROUPS OF MICE SUBMITTED TO EPIDEMIC INFECTION.

Infection.	Normal.		Vaccinated			
			I.P.		P.O	
	N.E.	S.	N.E.	S.	N.E.	S.
None	58·00	58·00	—	—	—	—
Mouse Typhoid (*a*) . .	22·49	34·75	—	—	—	—
Mouse Typhoid (*b*) . .	26·26	36·47	32·10 35·06	40·17 36·90	—	—
Mouse Typhoid (*c*) . . .	31·18	42·40	38·19	49·52	35·30	44·87
Pasteurellosis	22·37	37·39	—	—	—	—
Ectromelia (*a*)	30·71	54·69	—	—	—	—
Ectromelia (*b*)	20·8	52·8	49·1	51·6	—	—

I.P. = Intraperitoneal. P.O. = *Per os.*
N.E. = New Entrants. S. = Survivors after 30–100 days.

The significance of the figures is obvious. With such bacterial infections as mouse typhoid and pasteurellosis residence and survival in an infected herd increases resistance, as measured by expectation of life, but never raises it to the normal level. However long a mouse has lived in such a herd it is never indifferent to the risk that it continues to run. There is no solid immunity.

In the particular case of mouse typhoid, active immunization, by the intraperitoneal injection of two massive doses of a vaccine with an optimal antigenic character, will induce an increase in resistance of the same order as that attained after long residence in the herd. This increased resistance on entry is augmented by life in the infected herd; but vaccination followed by exposure to risk never raises the resistance to the normal level. Here, again, solid immunity is not attained. The effect of *per os* immunization is similar in kind to that of intraperitoneal immunization, though the resistance induced is slightly inferior.

In the case of the virus disease, ectromelia, the natural immunizing response is far more effective. The immunity attained is not complete—old survivors sometimes die of typical ectromelia, even though they have passed through a characteristic attack many months earlier—but it is of a high order. In conformity with this, vaccination with a formolized virus greatly increases the expectation of life of the mice exposed to risk.

It is clearly of interest to determine the effect of immunizing all entrants to an infected herd. Will this procedure result in the elimination of the disease, at least in its overt form? In the particular case of mouse typhoid, it will not.

In an experiment designed to test this point (Greenwood, Topley and Wilson 1931*c*) 3 normal mice were added daily to an infected herd from March 15th, 1929, to May 12th, 1929. The limited expectation of life on entry of these mice was $27{\cdot}00 \pm 1{\cdot}019$ days. From May 13th, 1929, to October 20th, 1929, 3 immunized mice were added daily instead of the 3 normal mice, and from October 21st, 1929, to June 29th, 1930, 1 immunized mouse was added daily to see whether slowing the rate of immigration would have any significant effect on the epidemic prevalence. The ${}_{60}E_x$ figure for the immunized mice added during the three-a-day period was $34{\cdot}92 \pm 0{\cdot}684$ days, for those added during the one-a-day period it was $36{\cdot}38 \pm 0{\cdot}938$ days—results very similar to those recorded above. But mouse typhoid was spreading and killing as actively in June 1930 as it was in March 1929. The mice at risk lived a little longer, but that was all.

It would, of course, be quite wrong to conclude—even if we may justifiably argue from mice to men—that active immunization of such a kind is useless. As has been noted above, it is capable of completely protecting a proportion of mice against a single experimental infection with a dose of living bacilli that is fatal to unvaccinated controls. Under conditions in which exposure to risk is slight, transient or intermittent, a resistance of this order may make all the difference between escape and infection, or between life and death. The lesson, so far as it is applicable, would appear to be that such resistance does not allow its possessor to ignore the risk of infection. We could not, by universal immunization of such a kind, stamp out a disease irrespective of the sanitary environment.

The Rôle of Bacterial Variation in Herd Infection and Immunity.

This problem, which may well be of major significance for the student of infective disease, is at the moment in a rather curious position. Judging from much of the current epidemiological literature one would assume that the primary question —Do changes in bacterial characters play an important part in epidemic happenings?—had been answered clearly and decisively on a basis of incontrovertible evidence. There is free reference to "epidemic" strains of particular bacteria, to loss and gain of virulence and infectivity during an epidemic prevalence, to secular changes in the pathogenic powers of a particular parasite. But nearly all such writing is really only the writer's way of saying that the disease in question was more prevalent, or more fatal, or spread more rapidly at one time than at another. The difficulty, which has seldom been faced, is that a description of epidemic events is, of necessity, a description of a fluctuating equilibrium between parasite and host. For an increase in bacterial virulence we can substitute a decrease in host resistance, or *vice versa*; the effect, *on balance*, is the same. If our descriptions are to be used as evidence in solving this particular problem the rise in virulence, or the fall in resistance, must be proved, not assumed; and the proof is no easy matter.

That changes in host resistance are quite certainly of primary importance will be obvious from our consideration of the natural history of diphtheria or scarlet fever in man, and of the happenings in herds of mice. It may also be conceded that the introduction into a community of a virulent strain of an organism with the production either of the carrier state or of manifest disease, may initiate an epidemic. It is less evident that changes in infectivity or virulence of the bacterial parasite are responsible for the fluctuations and other changes commonly observed during the course of the epidemic.

Of indirect and suggestive evidence there is plenty. We know that bacteria frequently undergo variation in the laboratory; and we know that avirulent or atoxigenic variants may be isolated from man or animals (see for instance Reimann 1927, Wadsworth and Sickles 1927, Okell 1929). We know also that antibodies acting on the surface antigens of the smooth virulent forms of bacteria are in many cases capable of inducing the S $\longrightarrow$ R variation (see for instance Griffith 1923). We know that many epidemic diseases have displayed wide secular fluctuations in their fatality. Taking our biological and epidemiological knowledge as a whole, the conclusion that bacterial variation plays an important part in epidemic happenings seems so probable that an experimental answer in the opposite sense would certainly be suspect—suspect to the extent that we should want to be very sure that our experiments had been properly planned, and that our conclusions had not been arrived at by an unjustifiable inductive generalization from a few particular instances. Experiment, combined with carefully controlled bacteriological and immunological observations in naturally occurring epidemics, must, of course, be our final court of appeal; but neither from this source nor from any other should we accept hasty judgments.

The experimental data at present available may be summarized as follows:

Webster and his colleagues (Webster 1923*a*, *b*, *c*, *d*, 1924*a*, *b*, *c*, *d*, 1925, 1926, 1927, 1928, 1930*a*, *b*, *c*, Webster and Burn 1926, 1927*a*, *b*) have shown quite clearly that different strains of the same bacterium, isolated at different periods of the same epidemic prevalence, may possess an approximately equal virulence as judged by direct inoculation tests, including inoculation into the stomach through an œsophageal tube. This, indeed, was their constant finding with the strains they examined. They conclude that changes in bacterial virulence play no part in the fluctuations in mortality that may be observed at different stages of a long-continued epidemic prevalence. These they would regard as due to changes in the average host resistance, and particularly to innate differences among the hosts at risk, or to seasonal or dietetic factors, that affect resistance in some non-specific way. They have, however, recorded differences in virulence among strains of the same bacterial species isolated from different epidemic prevalences of the same infective disease (Webster 1930*d*, Hughes 1930, Hughes and Pritchett 1930, Pritchett, Beaudette and Hughes 1930*a*, *b*), and they would regard these differences in virulence as determining certain observed differences in behaviour between one epidemic and another —for instance, differences in the proportion of carrier infections to fatal cases of the disease. More recently Webster and Clow (1933) recorded an interesting series of observations on the variations in the virulence of pneumococci for mice. They found that a strain of high virulence, as judged by intraperitoneal injection, might be of low virulence as judged by injection into the nasal cavity; and that a strain of high initial intranasal and intraperitoneal virulence might, as the result of passage from nose to nose, lose all, or almost all, its intranasal virulence, without affecting its intraperitoneal virulence. These findings did not, however, lead them to modify their earlier conclusions.

We have so far considered variations in *virulence*; but virulence may not be

the only factor concerned. If we assume the possible existence of another factor, labelling it *infectivity* and defining it as the *capacity to spread from one host to another under any specified conditions of exposure to risk of infection*, we may inquire whether infectivity is so highly correlated with virulence that we may regard them as synonymous terms.

The evidence available (Topley, Greenwood, Wilson and Newbold 1928) suggests, as would be expected, that those strains of *Salm. typhi-murium* that are capable of spreading rapidly among a herd of mice, giving rise to severe epidemics of mouse typhoid with a high mortality rate, are always of relatively high virulence as judged by direct intraperitoneal inoculation, and that strains of low virulence are incapable of giving rise to severe epidemics, or of spreading widely among the herd at risk, although they may persist for long periods in the tissues of the originally infected hosts, and may give rise to a few latent infections, or even to an occasional death, among the normal mice exposed to risk.

The correlation between virulence and the power to induce severe epidemics by contact infection does not, however, hold with all strains of *Salm. typhi-murium*. A particular strain of this organism, isolated originally from an infected mouse, was found to possess an unusual combination of characters (Topley, Greenwood and Wilson 1931). It was of moderately high virulence, killing in repeated tests some 65–75 per cent. of mice inoculated with 100–1,000 bacilli, but it possessed little power of producing severe epidemics by contact infection. In six closed epidemics, in each of which 100 normal mice were exposed to infection from 25 infected companions, the average limited survival time of the exposed mice ($_{60}E_x$) was 51·27 days—not far short of the normal limit. The proportion of the survivors harbouring *Salm. typhi-murium* in their spleens at the end of the period of observation was, however, high—an average of 31 per cent. for the six epidemics. Data were available for two other groups of epidemics, initiated with two strains of *Salm. typhi-murium* of low virulence as tested by direct inoculation. In one group the average $_{60}E_x$ was 52·62 days, in the other 52·21 days—figures almost identical with those obtained with the more virulent strain—but the percentage of infected survivors was 9·7 in one group and 9·0 in the other, less than one-third of the figure recorded for the relatively virulent strain. This strain had therefore the following combination of characters: a relatively high virulence when injected into the tissues, a considerable power of contact spread with the production of latent infections, but a relatively slight power of producing fatal contact infections.

It was of obvious interest to test the resistance to contact infection, with a highly virulent and infective strain, of survivors from these six epidemics of latent infection, and to compare it with the resistance of survivors from more fatal epidemics and with that of artificially immunized mice. An experiment carried out along these lines gave the results shown in Table 90.

TABLE 90

	Average $_{60}E_x$.	Per cent. Surviving on 60th Day.
Normal mice	37·49 ± 2·613	36·7
Vaccinated mice	41·95 ± 1·529	53·0
Survivors of severe epidemics	48·31 ± 1·836	66·0
Survivors of mild epidemics	48·75 ± 1·861	69·0

Thus the survivors from these mild epidemics were much more resistant than normal mice, significantly more resistant than vaccinated mice, and just as resistant as the survivors from epidemics of greater severity, involving a more stringent mortuary selection. The strain that gave rise to these mild epidemics might, perhaps, be regarded as one in

which the natural immunizing activity was greater than the natural killing power. Clearly, *if* a strain of this character made its appearance during an epidemic prevalence it *might* exert a significant influence on the course of events. But we cannot disregard the *if*.

In more recent experiments on epidemic pasteurellosis (Greenwood *et al.* 1936), results were obtained that seem to afford a satisfactory proof that changes in infectivity do occur during the epidemic spread of a bacterial parasite. Certain strains of *Pasteurella* that originally possessed little power of contact infection, though they were of moderately high virulence as judged by direct inoculation into mice, have, during their slow and limited spread in an experimental herd, given rise to variants in which the capacity for rapid and fatal spread was well developed. These experiments, like that recorded above, do not indicate a complete correlation between virulence and infectivity. If we adopt a term that is very commonly, though a little loosely, employed in current literature, and speak of an *epidemic strain* of a particular bacterial species, defining it as a *strain that has the capacity to spread naturally among a herd at risk giving rise to a severe and fatal epidemic*, we might hazard the guess that an epidemic strain is characterized by two attributes, high virulence and high infectivity, and that it may lose its epidemic character by losing either attribute. On what infectivity, as apart from virulence, depends we have as yet no knowledge. It does not depend primarily on an ability to multiply *in the tissues*: for a strain may be highly virulent but not highly infective. It must presumably depend on adaptations that enable it to meet those environmental changes that are associated with transfer from host to host, and to establish in the recipient host a primary focus of infection from which subsequent tissue invasion can occur. But to say this is merely to define our problem a little more closely.

In summary; variations in virulence in a bacterial parasite during an epidemic prevalence have often been sought for with negative results. There is, however, experimental evidence that different strains of the same bacterial parasite, possessing different degrees of virulence or of infectivity, produce very different results when they are allowed to spread by natural infection among a susceptible herd. There is also evidence that a bacterial parasite may vary in infectivity during an experimental epidemic. Taking the experimental evidence as a whole, it would seem to accord quite well with the view, expressed by many epidemiologists, that variations in the characters of the parasite are of major importance in the spread of human infections, and that an outbreak of disease may be initiated by the evolution, or importation, of an "epidemic strain" of the causative organism. Nevertheless, it seems likely that the evolution within any parasitic species of a strain of high epidemicity or virulence is an occasional event, rather than part of a normal or periodic process determining the fluctuations in mortality and morbidity that may occur during a long-continued epidemic.

The Rôle of other Factors in Herd Infection and Herd Immunity.

All those factors that affect the resistance of the individual will affect the average resistance of the herd of which he is a member. Those factors that have been adequately studied have been considered more or less briefly in earlier chapters.

There are, as we have said, special factors, such as the spatial distribution of the hosts at risk, the duration or the intermittency of contact, and other conditions of arrangement or environment, that affect herds but not individuals. Some of them, particularly those concerning the dispersal and aggregation of the infected and uninfected members of a herd, have been studied experimentally (Greenwood *et al.* 1936, 1939).

In one experiment, susceptible mice were added at a steady rate to an already infected

herd. With low rates of addition, up to 1 or 2 mice each day, the death rate showed wide and irregular fluctuations, with occasional intermissions. With higher rates of addition, the death rates showed minor fluctuations, without intermission, round an almost steady average value; in the circumstances the population increased rapidly at first, and later more slowly, suggesting that a still higher rate of addition would have produced a stable population in which the daily deaths equalled the daily additions. Thus, continuous contact of infected animals with susceptibles introduced into the herd at a steady rate did not produce a system with any inherent tendency to fluctuate, as epidemics fluctuate in natural conditions.

The effect of discontinuous contact was tested in another experiment. Twenty-five mice were infected with *Salm. typhi-murium*. These, and 100 normal mice were kept each in a separate cage. Three times weekly, all the mice were herded in one large cage for 4 hours and at the same time 2 normal mice were added to the herd. An epidemic started among the normal mice, but soon died down, and after 70 days, there were no deaths from mouse typhoid. By the 149th day, the population of mice had increased to 180, and there had been no deaths for 80 days. The population was then aggregated in a single cage. An epidemic wave resulted from this continuous contact, and in spite of the addition of 6 new mice per week, the total population had fallen by the 289th day from 180 to 44 mice. The resumption of discontinuous contact at this time was followed by a prompt response in the reverse direction. The death rate sank and remained at a low level, and the population rose to over 100 mice. These conditions were maintained by intermittent contact over a period of 15 months, at the end of which re-aggregation again produced a major epidemic, which within 30 days reduced the population to 20.

The effect of dispersal upon the course of an experimental epidemic was found to depend on the degree of dispersal and the age of the epidemic. Thus the dispersal of 100 mice into four groups of 25 had little effect upon an epidemic that had just begun to spread. If the mice were divided into smaller groups as, for example, 10 groups of 10 mice, the subsequent mortality was much lower than that of a control group of 100 maintained as a single unit. When dispersal was delayed until the epidemic was well under way, it had little effect, even though the dispersed groups were small (Topley 1922, Topley and Wilson 1925, Greenwood *et al.* 1930). These are results with epidemics in closed herds. The effect of dispersal was also tested in an infected herd in which a continuous spread of infection was maintained by the addition of susceptible mice in large batches at short intervals. Mice were withdrawn to isolation in single cages, after varying intervals of exposure to infection, and the rates of infection and mortality compared with those of added mice left in the herd. The average mortality of mice subsequent to withdrawal was lower than that of their contemporaries left in the herd. The advantage of withdrawal was marked for all periods of exposure up to the longest tested, which was 35 days. The greatest effect, as would be expected, was observed in mice withdrawn after the shortest exposure (Greenwood *et al.* 1939).

The conclusions to be drawn from these experiments, though they must in some cases be tentative, add to the evidence that has accumulated from the study of natural epidemics in man and animals, and point to the fact that movements of susceptible and infected hosts in relation to one another, and the aggregation or dispersal of human or animal herds, apart from any introduction of new infection, are sufficient to induce major changes in the incidence of many infective diseases (Topley 1942; see also McKendrick 1940). Many other factors have been studied in far greater detail but with less control under field conditions. Some day we may be able to work these factors into our immunological picture in terms of the effects produced by changes in the rate at which infection is received, or in the rate at which immunization proceeds (see for instance Dudley 1923); but that day has not come yet.

The Control of Herd Infection. The study of herd infection and herd immunity by the methods described above is as yet in its infancy but it has already modified many of our conceptions with regard to the administrative control of epidemic disease. Isolation and quarantine, for instance, have, in the past, played a major part in public health administration. A very cursory consideration of the various types of distribution of a bacterial parasite within an infected herd that have been described earlier in this chapter, and of the results obtained in experimental epidemics, will raise serious doubts as to the probable efficacy of such measures. In the case of quarantine, it is clear that it can succeed only where it is complete ; and the history of sanitary control suggests that successful quarantine is possible only under very exceptional conditions, and over relatively short periods of time. The carrier and the atypical case defeat a sanitary barrier, just as they defeat isolation ; and, under the conditions of modern transit, there seems little possibility of preventing the introduction into any one part of the inhabited world of any infective parasite which is prevalent in another. Once the barrier is passed, the subsequent course of events will depend upon the conditions obtaining within the community into which infection has been introduced.

The policy of the isolation of sick persons within a community is based on a similar failure to realize that the clinical picture provides a very incomplete description of the true state of affairs. If isolation removed from the community the whole, or even the great majority, of the infected individuals, it might be expected to exert a considerable influence on the prevalence of an infective disease. But if the ratio of latent or atypical infections to clinically recognizable cases is high, we cannot hope to effect any marked reduction in the morbidity rate by removing to hospital those cases which exhibit the typical stigmata of the disease. We may indeed effect a smaller reduction in the total infective material than the ratio of isolated to non-isolated, among infected individuals, would suggest ; for the sick person would, in any case, move less freely among his fellows than the apparently healthy carrier. As in the case of quarantine, we should expect a policy of isolation to be successful only in exceptional circumstances, when the recognizable cases form a very high proportion of the total infected, and when the total mass of infection to be dealt with is small. Once a given infective disease has assumed an endemic-epidemic prevalence within a herd, we should expect no appreciable result from the isolation hospital, so far as a reduction in morbidity is concerned. It is interesting to find that the expectations based on bacteriological and experimental findings are borne out by administrative experience. Thus, an attempt has been made, by an analysis of the available records, to answer the question : Does hospital isolation have any effect upon the incidence of scarlet fever ? All applications of the calculus of correlations have wholly failed to bring out any connection whatever between the incidence rate of scarlet fever and the extent of isolation (see Greenwood and Topley 1925). The value of the isolation hospital must apparently be judged by the benefit which it confers on the sick within its walls ; for it would seem to have little effect on the health of the community as a whole.

Turning to those administrative measures which bring about a general reduction in the opportunities for the transference of infection from host to host, theoretical considerations suggest that action along these lines is likely to be effective. Any improvement in sanitation, which ensures a clean water, or milk, or food supply, which reduces the frequency of insect vectors of infection, or which lessens the opportunities for close contact between individuals, may be expected to lead to

a reduction in the incidence of those infections, with the transference of which that particular measure of sanitation interferes. This expectation has been fulfilled whenever an effective method of reducing the general opportunities for the transference of a particular infection has been adopted.

It may be noted that it is in the case of infections of the respiratory tract that it is particularly difficult, under modern conditions of civilization, to devise any generally applicable and effective means of preventing transit of the parasite from host to host, and that it is exactly these diseases which have so far largely defied the attacks of preventive medicine (see Chapter 91).

Finally, the whole trend of our present knowledge stresses the importance of the susceptible host. Any measure which increases the average resistance of a herd, whether it depends on specific immunization, or on some factor which confers an increased immunity less specific in its range, will exert a direct influence on the incidence of the corresponding infections. The accurate determination of the conditions which must be fulfilled, in order that induced variations in immunity should exert their optimal effect, and the development of methods by which such immunity can be induced, are two of the most important problems which await solution.

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PART IV

THE APPLICATION OF BACTERIOLOGY TO MEDICINE AND HYGIENE

CHAPTER 57

ACTINOMYCOSIS, ACTINOBACILLOSIS, AND RELATED DISEASES

Introductory

The term Actinomycosis was originally given by Bollinger in 1877 to a disease in cattle characterized by the formation of a hard wooden swelling of the tongue or a fusiform enlargement of the jaw. In the lesions of this disease he found numerous opaque slightly yellowish bodies of coarsely granular or mulberry-like appearance, which were found to consist of a fungus. His colleague Harz, a botanist, to whom he referred for an opinion on this organism, decided that it was a true mould and suggested for it the name of ray fungus or *Actinomyces bovis*. A similar mycotic disease in man was described the following year by Israel (1878, 1879); and in 1879 Ponfick suggested that the two diseases were identical. From seven cases of actinomycosis in cattle, Bostroem in 1891 isolated an aerobic filamentous organism showing true branching; and from two cases in human beings, Wolff and Israël in the same year (1891) isolated a similar branching organism, which was, however, anaerobic. In 1902 Lignières and Spitz described a new disease in cattle, differentiated from actinomycosis chiefly by its tendency to invade the lymph glands. From the affected animals they cultivated a very small, non-branching, Gram-negative bacillus, to which they gave the name actinobacillus. The disease they called actinobacillosis.

As early as 1874 Vandyke Carter drew attention to a disease in India known as Madura foot. Microscopical examination revealed the presence of a fungus, but attempts to cultivate it were unsuccessful till 1894 when Vincent succeeded in isolating an aerobic branching filamentous organism, similar in many respects to *Actinomyces bovis*, which he called *Streptothrix maduræ*.

From the disease known as "Farcy of cattle," Nocard in 1888 cultivated an aerobic branching filamentous organism, which likewise resembled Bostroem's *Actinomyces*. Similar branching organisms have been encountered in a number of diverse lesions in human beings and other animals.

From this brief description, it will be realized that there are several diseases from which actinomyces-like organisms have been isolated. In one group of diseases, their proliferation occurs in the form of colonies which have a definite ray structure; such colonies are seen in the pus as granules or *Drusen*. In

another group the organisms form a felted mycelium without any radial arrangement, and without any formation of granules. Some authors prefer to limit the term actinomycosis to diseases of the former group, denoting those of the latter group by such terms as Streptothricosis, Cladothricosis, Oosporosis, Pseudotuberculosis, Para-actinomycosis, or Pseudo-actinomycosis. In Chapter 14 we have given our reasons for including all the filamentous branching organisms in the *Actinomyces* group. The logical sequence of this is to classify all diseases caused by these organisms under the name of Actinomycosis. This term, however, is often used in a broad sense to include chronic granulomata characterized by club formation. Such granulomata are in fact sometimes caused by *Staph. aureus* or *C. pyogenes*, and though their inclusion may be justified on clinical grounds, it leads to great confusion bacteriologically. We therefore propose to exclude from Actinomycosis the granulomatous lesions of cattle and swine that are caused by organisms other than those belonging to the *Actinomyces* group. The staphylococcal lesions are better referred to as Botriomycosis (see Chapter 67).

ACTINOMYCOSIS.

A. Due to *Actinomyces bovis* of Wolff and Israël. True ray-fungus disease in man and cattle. Granules or *Drusen* formed in tissues. Anaerobic.

B. Due to *Actinomyces*—aerobic types.
   (1) Non-acid-fast group. Madura foot—granules found in tissues. Other lesions in man and animals—no granules found in tissues.
   (2) Acid-fast group—chiefly pulmonary and abdominal infections in man. No granules formed in tissues. Farcy of cattle—no granules formed in tissues.

C. Due to *Actinomyces muris*. Rat-bite fever (one type) in man, and infective arthritis in mice. No granules formed in tissues.

ACTINOBACILLOSIS.

Due to bacilli.
   (1) *Actinobacillus lignieresi* of Lignières and Spitz. Actinobacillosis in cattle. Granules or *Drusen* formed in tissues.
   (2) *Actinobacillus actinoides* of Theobald Smith. Broncho-pneumonia of calves: pneumonia of rats. No granules formed in tissues.

ACTINOMYCOSIS

A. Due to Actinomyces bovis Wolff and Israël

Epidemiology.—Actinomycosis in man is not a common disease. According to van der Hoeden's (1939) observations in the Netherlands, it is three times as common in males as in females, and ten times as common in rural districts as in large towns; about 80 per cent. of the cases occur in patients over 20 years of age. The cervico-facial region is the commonest situation attacked. Table 91, which we have compiled from cases observed by Illich and by Leith (see Delépine 1915), and Colebrook (1921), indicates the distribution of the lesions. Cope (1938) has collected a rather larger series of cases in his monograph on actinomycosis, but the percentage distribution is much the same.

The mortality of the disease depends largely on the site affected. Of 10 cases of cervico-facial infection observed by Colebrook (1921), 9 recovered: on the

other hand 12 out of 14 thoracic and abdominal infections proved fatal. Of 87 cases recorded by Harbitz and Gröndahl (1911), 42 died or remained unhealed.

TABLE 91

DISTRIBUTION OF ACTINOMYCOTIC LESIONS IN MAN.

	Cases.	Percentage.
Head and neck	234	51·4
Tongue	16	3·5
Abdomen	99	21·8
Thorax	65	14·3
Skin	11	2·4
Other situations and doubtful	30	6·6
	455	100·0

The extent to which *cattle* are infected is difficult to ascertain, largely because the disease has so frequently been confused with actinobacillosis. According to Jelenevski (see Hutyra and Marek 1926) the morbidity, as estimated in various abattoirs from 1896 to 1911, was as follows: Berlin 0·31 per cent., Vienna 0·01 per cent., Moscow 3·34 per cent., Kief 0·67 per cent., and Warsaw 0·65 per cent.; but the mild form of lingual actinomycosis was apparently not included in these statistics. Even in the same country, the frequency appears to vary from one part to another. Thus, in the Netherlands, van der Hoeden (1939) gives the incidence of lesions in slaughtered cattle as ranging from 0·42 per 1,000 in Gouda to 12·17 in Amsterdam. Of 85,445 tongues imported from the Argentine and examined by MacFadden (1913) in London from August to October 1913, 4,949 or 5·8 per cent. showed lesions, but there is evidence to suggest that many of these were infected with the actinobacillus. In this country the disease is not infrequent. It is stated to be commoner in low-lying marshy districts, and to affect stall-fed cattle more frequently than those fed on pasture. According to Magnusson (1928), it affects almost exclusively the jaw bone, which develops a typical fusiform swelling; but Albiston and Pullar (1934) state that the soft tissues—tongue, lymph and salivary glands, muscle—may be affected. Of 1,015 cases observed in abattoirs in the Netherlands, 900 affected the head, 61 the lungs, 11 the abdominal organs, 32 lymph glands, 10 the udder, and 1 the foot (van der Hoeden 1939). Generalization of the infection is not uncommon in man, but is rarely seen in cattle. So-called actinomycosis of the udder in cattle seems to be almost invariably due to staphylococcal infection (Magnusson 1928, Albiston 1930).

Actinomycosis is by no means infrequent in *swine*. The udder is attacked. Magnusson (1928) states that two types of *Actinomyces*, both anaerobic, may be found, which differ from the *bovis* variety. Some cases are due to staphylococci, and are more correctly termed botriomycosis. The disease occurs rarely in sheep, goats, and dogs. Most carnivora are resistant. We have met with one case in a mouse, in which the liver showed typical actinomycotic lesions.

Mode of Infection.—The mode of infection both in man and animals is obscure. In the days when Bostroem's organism was held to be the cause of the disease, it was generally thought that infection was conveyed by grasses, which lodged in the mucosa and provided a suitable nidus for the development of the organism. This

view appeared probable because the organism was found to lead a saprophytic existence outside the body, being frequently isolated from grasses and cereals, and because awns of grasses, or barley spikes, are not uncommonly found in the actual lesions. But now that Bostroem's organism is regarded as an accidental contaminant, and the organism isolated by Wolff and Israël as the true cause of the disease, this hypothesis has been abandoned. Wolff and Israël's organism appears to be a strict parasite; it has never been found outside the animal body, and its survival in artificial culture is brief. These facts suggest that the disease is transmitted by infected pus, saliva, or nasal discharge.

Strong evidence in favour of this view is provided by the work of Naeslund (1925, 1926, 1929) who, in a study of the flora of the human mouth, succeeded in isolating both aerobic and anaerobic types of *Actinomyces*. The anaerobic types were indistinguishable from *Actinomyces bovis*, and were more frequently met with than the aerobic types. They were, moreover, isolated from a high proportion of salivary calculi, thus supporting Söderlund's (1921) view that the formation of these calculi must be regarded as a specific result of actinomycotic infection. The same organisms also seemed to play an important part in the development of dental tartar. Bibby and Knighton (1941) regard Naeslund's micro-aerophilic and anaerobic strains as probably belonging to the *Leptothrix* group; but the work of Lord and Trevett (1936), Emmons (1938), Sullivan and Goldsworthy (1940), and Slack (1942) seems to leave little doubt of the occurrence of true *Actinomyces* strains, indistinguishable from the Wolff-Israël type, in the human mouth, notably in diseased tonsils, carious teeth, and pyorrhœal pus. If this organism is accepted as an inhabitant of the human mouth, the factors predisposing to the development of actinomycosis have still to be determined. Possibly suitable necrotic foci, such as septic teeth, have to be present in which the organisms can develop under anaerobic conditions. There are records of three cases in which the disease followed a human bite (see Robinson 1944).

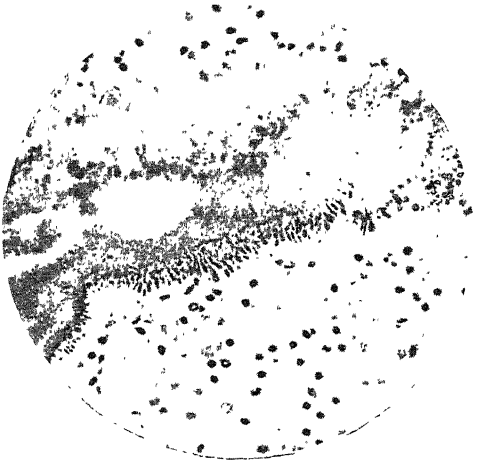


FIG. 269.

Part of the edge of a colony of *Actinomyces bovis* in the jaw of an ox, showing the peripheral zone of clubs (× 350).

Bacteriology.—In the tissues *Actinomyces* grows in the form of colonies or *Drusen*, which macroscopically have the appearance of small granules. A section suitably stained reveals the presence of a central mass of partly necrotic material, in which branching filaments may be recognized, and a peripheral zone of swollen bodies looking like clubs and arranged radially. The clubs are surrounded by pus cells, outside of which there are aggregations of mononuclear cells and dense masses of fibrous tissue. The colony often presents a characteristically scalloped edge. The mode of formation of

the clubs is still under dispute, some workers maintaining that they represent the swollen extremities of the filaments, others that they are due to a reaction on the part of the tissues. The fact that they occur in so-called actinomycotic lesions due to staphylococci, and that they may be formed under experimental conditions around dead masses of tubercle bacilli and actinomyces, suggests that the second explanation is more probably correct. The disease spreads by contiguity; the surrounding tissues, no matter of what type, are invaded and destroyed.

The pus discharged from these lesions is not usually abundant; it is yellow, often bloody, thick or thin, and sometimes tough and viscous. If shaken up with water in a test tube it will be seen to contain small granules, which rapidly sink to the bottom. They vary greatly in size, but are generally about 0·25 to 1 mm. in diameter; they are opaque, spherical bodies, greyish-white by reflected and yellowish by transmitted light, and have an oily appearance. Generally they are soft and can be readily crushed, but in cattle they may be hard or even calcified. In pus from old lesions they are darker in colour, and not infrequently brown. Microscopical examination of these granules reveals the presence of a felted mycelium, together with rod-shaped and coccoid bodies, surrounded by radially disposed clubs. The filaments are irregularly Gram-positive; the clubs are usually Gram-negative. Clubs are said to be more conspicuous in lesions of cattle than in those of man.

Though all observers are agreed on the microscopical appearance of the organism in the tissues, attempts to cultivate it have led to divergent results. Bostroem in 1891 was the first to report positive results. By inoculating several hundred tubes of medium from a few cases of actinomycosis in cattle he succeeded in obtaining about 12 cultures of an aerobic, branching, pigmented, filamentous organism. Although he was unable to reproduce the disease in animals by injection with this organism, he had no hesitation in concluding that it was the cause of the disease. A little later in the same year Wolff and Israël (1891) reported the isolation of an anaerobic, branching, filamentous organism from 2 cases of human actinomycosis. (For a description of these organisms see Chapter 14.) Attempts to reproduce the disease experimentally in laboratory animals with this organism were not very successful; at the most, small, apparently retrogressive, granulomatous tumours resulted from inoculation. In 1905 Wright in Boston carried out a more thorough investigation. From 13 cases of actinomycosis in man and from 2 in cattle he isolated in pure culture an organism of the Wolff-Israël type, but like his predecessors was unable to produce progressive disease by animal inoculation. From a critical résumé of the literature, however, he reached the conclusion that the Wolff-Israël organism was the true cause of actinomycosis. Bostroem's organism he regarded as an example of the numerous saprophytic fungi which are common on grains and grasses, and which are therefore liable to gain access as contaminants to actinomycotic lesions.

Subsequent work tended more and more to confirm Wright's conclusions. The observations of Henry (1910), Colebrook (1920) and Bosworth (1923) in this country, of Harbitz and Gröndahl (1911) in Norway, and of Magnusson (1928) in Sweden left little doubt that the Wolff-Israël type was the principal organism responsible for actinomycosis in man and cattle. Colebrook showed that the serum of severely infected human patients might agglutinate this organism; and Magnusson found that, though the disease could not be satisfactorily reproduced

in small animals, it was possible to set up typical actinomycotic lesions by the inoculation of pure cultures into cattle. That it has not been cultured from all cases of the disease is probably explained by technical difficulties, especially when the lesion is invaded by secondary organisms, and by the not infrequent sterility of granules from old lesions.

We may conclude that the evidence in favour of the ætiological rôle of the *Actinomyces bovis* described by Wolff and Israël is sufficient to justify us in accepting this organism as the cause of that variety of actinomycosis which is characterized by the presence in the tissues of the typical ray fungus.

One further point in connection with the bacteriology of the disease may be mentioned. In the characteristic granules it is not unusual to encounter, as well as the branching filaments of *Actinomyces*, densely packed masses of Gram-negative cocco-bacilli. These organisms were first described and isolated by Klinger in 1912 who named them *B. actinomycetem comitans*; and they have been subsequently noted by Colebrook (1920) in this country, and Bayne-Jones (1925) in America. What relation these organisms bear to *Actinomyces bovis* is not clear. (For further information see Chapter 14.)

Diagnosis, Prophylaxis and Treatment.—Diagnosis is made by microscopical and cultural examination of the affected tissues. The granules should be crushed between slides and stained by Gram's method. If calcified, they should first be treated with hydrochloric acid. The presence of a mycelium of Gram-positive filaments surrounded by radially disposed Gram-negative clubs is characteristic of actinomycosis. Cultures are made from fresh young granules only; after thorough washing, they are seeded into a number of tubes containing blood broth (Gordon 1920), glucose agar, or serum agar; incubation is carried out under both aerobic and anaerobic conditions, preferably in an atmosphere to which 5–10 per cent. CO_2 has been added. Rosebury, Epps and Clark (1944) prefer to streak the unwashed granule or the pus on to four plates in series of Bacto brain heart infusion agar and to incubate in an anaerobic atmosphere to which 5 per cent. CO_2 is added. Primary cultures are frequently contaminated; single colonies should therefore be picked off to obtain pure cultures. Examination of the agglutinating power of the patient's serum to the organism isolated should be carried out if possible. In pulmonary actinomycosis in man the sputum is tough, often viscous, and sometimes hæmorrhagic; the typical granules can be found on examination (Harbitz and Gröndahl 1911). Most of the abdominal cases commence in the appendix. In the rare cases of actinomycotic meningitis, the primary lesions are generally in the lungs and bronchial glands (Henry 1910).

As we are still ignorant of the factors determining the development of the disease, it is difficult to lay down prophylactic measures against it. It is clear, however, that the discharge from the lesions should be considered dangerous, and every care taken to prevent its coming into contact with man or other animals.

Colebrook (1921) reports favourably on vaccine therapy, provided efficient surgical drainage of the affected tissues is secured. Negroni (1937) claims to have obtained promising results with a formolized filtrate of broth cultures of parasitic aerobic strains of *Actinomyces*, employed presumably to stimulate a non-specific protein reaction. Penicillin treatment now seems to hold out greater promise of success.

B. Infections due to, or associated with, the Aerobic Members of the Actinomyces Group

(1) Madura Disease.

This disease, which is peculiar to man, was first thoroughly investigated by Vandyke Carter of Bombay in 1874. It occurs chiefly in India, but cases have been reported in Algiers, Italy, Roumania, America, and other countries. The lesions are commonest in the foot, and are of a chronic granulomatous nature, characterized by swelling, suppuration, and sinus formation. The pus contains granules, which in some cases are yellowish in colour, in others black. These granules are generally larger than those seen in actinomycosis—up to 1 mm. in diameter; they may be aggregated into masses looking not unlike fish-roe (Kanthack 1893). Under the microscope, those from the pale or ochroid variety of the disease consist of a central irregularly staining felted mycelium, showing true branching; sometimes they are surrounded by a ray formation of club-like structures. Vincent (1894) cultivated from them an aerobic filamentous organism, which gave a pink or rose coloration on potato (see Chapter 14). It proved to be practically non-pathogenic for laboratory animals. He called it *Streptothrix maduræ*; its correct name is *Actinomyces maduræ*.

Wright (1898) found that the granules from the black or melanoid type of the disease consisted of a mycelium of hyphæ or fungoid elements, more or less degenerated, embedded in a hyaline brown-coloured refringent substance, which itself formed more or less of a reticulum. The pigment could be dissolved by sodium hypochlorite solution, leaving the mycelium unaltered. Cultures resulted in the development of a mould, consisting of a branching septate mycelium, and forming typical mould-like colonies. Wright concludes that the pale variety of the disease is due to an organism of the *Streptothrix* (*Actinomyces*) group, while the black variety is due to a mould or hyphomycete. This view is supported by the observations of Tribedi and Mukherjee (1939). It is probable that several types of organism are responsible for the disease, and that the pale variety, at least, may be caused either by an *Actinomyces* or by a fungus.

(2) Other Lesions in Man.

Eppinger (1891) described a case of brain abscess in a glass-grinder due to a branching filamentous organism. Old calcified abscesses were found in the bronchial and supraclavicular glands, and pseudotubercles were noticed in the lungs and pleura. The organism grew abundantly on ordinary media under aerobic conditions (see Chapter 14). Injection into rabbits and guinea-pigs proved fatal in 1 to 4 weeks; post mortem the lungs, liver, and spleen were studded with small white nodules. He called the organism *Cladothrix asteroides* (*Actinomyces asteroides*) and the disease Pseudotuberculosis cladothrichica. Later MacCallum (1902) recorded a case of peritonitis in a negro child due to infection with the same organism. According to Claypole (1913) this organism is mildly acid-fast.

Henrici and Gardner (1921) isolated a similar organism from the sputum of a woman, which differed, however, in certain particulars, and which they called *Actinomyces gypsoides*. From the literature they were able to gather accounts of 26 cases of infection with acid-fast *Actinomyces*. Infection probably occurs by inhalation; the first lesions are in the peribronchial nodes. Later, caseous broncho-pneumonia develops, followed by central softening and cavity formation. Metastases may occur. The duration of the illness is generally about 6 months.

These cases are liable to be confused with tuberculosis. Goldsworthy (1937) recommends that the organisms should be sought for in the sputum of all patients with obscure or unusual chronic infections of the lungs, especially when repeated attempts to demonstrate the tubercle bacillus have failed. The organisms are liable to be decolorized in the ordinary Ziehl-Neelsen process, but their filamentous morphology is rendered strikingly evident by Gram's strain. Cultivation of the organism, and the exclusion of tuberculosis by inoculation of the sputum into guinea-pigs, should effect a differential diagnosis.

Actinomyces of the aerobic non-acid-fast type have been described in various lesions by numerous authors, such as in the bladder and prostate of a man with pyuria by Cohn (1913), in the purulent cornea by Namyslowski (1912), in the cerebro-spinal fluid of children with meningitis by Gerbasi (1927), and in a fatal infection of the lungs by Biggart (1934).

(3) **Cattle Farcy. Farcin du bœuf.**

Cattle farcy is characterized by the appearance—generally on the medial surface of the extremities—of firm, painless perivascular nodes, which later suppurate. When incised, they discharge a whitish, odourless mass resembling soft cheese (Report 1913). The regional lymph glands become converted into firm, painful tumours. It is a chronic disease lasting for a year or more, and marked in the later stages by severe cachexia. From the pus of an affected animal Nocard (1888) isolated an aerobic branching organism, now known as *Actinomyces farcinicus*. Intravenous injection of pure cultures into a cow and a sheep resulted in the development of miliary nodules. The disease is not common in Europe; it is very prevalent in Guadeloupe, and has been recorded in India, and in Kenya (Daubney 1927).

(4) **Other Lesions in Cattle.**

Evans (1918) has recorded an infection of the udder of cows with an organism of the aerobic *Actinomyces* group. She succeeded in isolating it from 18 out of 21 samples of milk, in which it was present in considerable numbers.

(5) **Goats and Horses.**

Silberschmidt (1899) isolated a member of this group from three goats; this organism is called *Actinomyces capræ*. The same organism was later cultivated by Galli-Valerio from the lung of a goat. Dean (1900) cultivated a member of the group from an abscess in the submaxillary region of a horse.

C. Infections due to, or associated with, the Facultative Aerobic Members of the Actinomyces Group.

(1) **Rat-bite Fever.**

For many years there has been great confusion over the ætiology of this disease. Schottmüller (1914), Blake (1916), Tileston (1916), and others described the isolation of a streptothrix—*Streptothrix muris ratti*—from the blood of patients suffering from rat-bite fever, while Japanese workers (see Chapter 82) brought strong evidence to show that the disease was due to a spirochætal organism known as *Spirochæta morsus muris* or *Spirillum minus*. There seems to be no question now that both organisms may be responsible, and that rat-bite fever may be of at least two different types. The evidence for this conclusion is based on a careful study of the literature.

In 1925 Levaditi, Nicolau, and Poincloux in France described an organism, to which they gave the name *Streptobacillus moniliformis*. It was isolated from the blood of a laboratory worker who was suffering from an acute disease characterized by fever, sore throat, papular erythema, and multiple arthritis. The following year Parker and Hudson (1926) gave an account of an epidemic disease occurring in Haverhill, Massachusetts. This disease they referred to as Erythema arthriticum epidemicum or Haverhill fever. An organism was isolated—*Haverhillia multiformis*—which was subsequently proved to be identical with *Streptobacillus moniliformis*. In neither instance was there any suspicion of infection having occurred through rats. In 1933 Strangeways accidentally encountered the same organism in mice that had died after intraperitoneal inoculation with the blood of rats infected with *Trypanosoma equiperdum*. Investigation showed her that *Streptobacillus moniliformis* was an inhabitant of the nasopharynx of the rat, in which it normally led an apparently harmless existence. A very similar organism had already been reported by Tunnicliff (1916) in the lungs of white rats affected with bronchopneumonia. It would therefore appear that *Streptobacillus moniliformis* is no other than the *Streptothrix muris ratti* of the earlier workers. Both organisms appear to be closely related to the *Actinomyces* group, and we have therefore suggested for them the name *Actinomyces muris*. Since this organism is pathogenic for man and is a normal parasite of the rat, it is not difficult to understand how it may be responsible for human infection. *Spirillum minus* is also a normal parasite of the rat, and may likewise cause rat-bite fever. We shall restrict ourselves at the moment to the description of the type of disease caused by *Actinomyces muris*. For the type caused by *Spirillum minus* see p. 1838

Rat-bite fever due to *Actinomyces muris* is an irregularly relapsing, febrile, septicæmic disease characterized by metastatic arthritis and morbilliform and petechial cutaneous eruptions. The incubation period is usually under 10 days, and may be as short as 3 days. The disease is often ushered in by a chill and a rapid rise of temperature. The wound usually heals without exacerbation. There is extreme prostration, severe generalized muscular pain and tenderness, headache, weakness, and a widespread morbilliform or petechial eruption. Generalized enlargement of the lymph glands may occur. Non-suppurative arthritis, affecting one joint after another, is a characteristic feature of the disease. There is a leucocytosis with a high proportion of neutrophiles. After a few days the temperature falls by crisis, and the disease then assumes a relapsing type, febrile paroxysms occurring at irregular intervals for weeks or months. The case mortality is not known with certainty, but a figure of 10 per cent. may be quoted. Little is known of the post-mortem appearances, but Blake's case showed an ulcerative endocarditis and a subacute myocarditis. Diagnosis during life is best made by cultivation of the causative organism from the blood stream during a febrile attack. About 20 ml. of blood are distributed partly into serum broth and partly over a number of plates of Loeffler's serum. The cultures may be incubated aerobically or anaerobically, preferably in the presence of 5 per cent. CO_2. According to Brown and Nunemaker (1942) agglutinins may be demonstrable in the patient's blood serum in a titre varying between 1/160 and 1/5,120. A suitable antigen may be prepared by formolizing a broth culture of an old laboratory strain of *Act. muris* that forms a homogeneous sediment. Arsenic is worth trying in treatment, but its real value is unknown. According to Heilman's (1940) experimental observations on mice, it is without effect, whereas gold sodium thiomalate protects

the animals against rapidly fatal doses of *Streptobacillus moniliformis*. Though infection usually follows a rat-bite, the outbreak of Haverhill fever studied by Parker and Hudson (1926) was traced to the consumption of raw milk, as was also another outbreak affecting 86 persons described by Place and Sutton (1934). How the milk becomes contaminated it is difficult to say, but the excretion of the organism in the urine of infected mice and perhaps rats affords a possible explanation. The differential diagnosis of rat-bite fever due to *Actinomyces muris* from that due to *Spirillum minus* has been studied by Allbritten, Sheely, and Jeffers (1940). The actinomycetal type has an incubation period usually under 10 days, the wound does not generally become inflamed when the temperature rises, the febrile paroxysms are less regularly periodic, the rash is of the small macular or petechial type, arthritis is a prominent feature of the disease, leucocytosis is common, and the effect of arsenic in treatment is doubtful. In the spirillar type, on the other hand, the incubation period is usually longer than 10 days, the local wound becomes inflamed when the temperature rises and may suppurate, the febrile paroxysms are more regular, the rash is of the large macular or papular type, arthritis is rare, leucocytosis is less common, and arsenic is specific in treatment. Both diseases apparently respond to penicillin treatment (Heilman and Herrell 1944). Reference, however, to the excellent review by Brown and Nunemaker (1942) suggests that, though some symptoms are commoner in one form of the disease than the other, both organisms may give rise to the same clinical picture.

(2) **Infective Arthritis of Mice.**

The natural disease produced in laboratory mice by *Actinomyces muris* has been described by Levaditi, Selbie, and Schoen (1932) and Mackie, van Rooyen, and Gilroy (1933), and in wild mice by Williams (1941). It may occur in either sporadic or epidemic form, and may be of an acute septicæmic or a more chronic polyarticular type. The mortality is high. Infection probably results either through bites, or through contamination with urine, which may contain the organism. In acute cases the animal appears ill and has a lustreless coat. The eyes may be glued together by a semipurulent conjunctival discharge. Death occurs in a few days, and nothing characteristic is found at necropsy. The subacute or chronic type is characterized by polyarthritis and myocarditis, the former evidenced by swelling of the joints, the latter by cyanosis of the tail and extremities. One of the commonest manifestations is œdematous swelling of the feet and legs, sometimes confined to one or both hind limbs. Ulceration of the feet may occur, but gangrene is rarely observed. Paralysis of the hind limbs, enlargement of lymphatic glands, subcutaneous swellings, and keratitis are other manifestations that are not infrequently encountered. The conjunctivitis may suggest infection with *Ery. murisepticа*, the swelling and ulceration of the feet ectromelia. Diagnosis is made by culture. The organism can often be isolated from the blood, even in chronic cases, and from the spleen, liver, glands and joints. Serum agar or Lœffler's serum should be used. Experimental reproduction of the disease in normal mice is readily accomplished by inoculation of pure cultures (see p. 386).

ACTINOBACILLOSIS

In 1902 Lignières and Spitz described under the term actinobacillosis a disease that had occurred in epizootic form amongst cattle in the Argentine during the summer of 1900–01. Once a herd had become infected the disease spread rapidly, attacking 15–25 per cent., sometimes even 50 per cent., of the animals in the course of a few weeks. After a short time the epizootic died down, but sporadic cases, generally of a mild form, occurred at intervals. The main animals attacked were cattle; sheep were rarely, and horses never attacked. In cattle all ages were affected, from calves of 1 month to animals several years old.

In about 80 per cent. of the cases the lesion was in the subcutaneous tissue of the neck; in about 5 per cent. in the tongue. With the exception of the lungs, the internal organs were not often affected. The most striking difference from actinomycosis lay in the frequency with which the regional lymphatic glands were invaded. In the neck the lesion commences as a rounded subcutaneous nodule of fibrous consistence, adherent to the skin, with a smooth but later protuberant nodular surface. During the next few days the lesion grows rapidly, softens in the centre, and becomes converted into a cold abscess. In this state it persists for weeks or months, gradually enlarging. Eventually it ulcerates and discharges a highly viscous, milky-white or slightly green, almost odourless pus, containing little greyish-white opaque granules up to 0·4 mm. in diameter. The pus is so viscous that it is rarely discharged completely; it forms crusts on the surface of a fungating ulcer, which persists indefinitely. The abscesses are usually multiple, and frequently occur in different situations simultaneously—such as the tongue, glands, and lungs. Fibrous tissue is formed around the lesions—as in actinomycosis. On examination the granules are seen to be simple or composite. They consist of tufts of radiating clubs varying in size from about $16 \times 8\ \mu$ to $99 \times 24\ \mu$. They are stained best by glycerol picrocarmine; in a few seconds the clubs take on a yellow colour, while the pus appears pink. No mycelium is found. If, however, the granule is stained with carbol-fuchsin, it is sometimes possible to distinguish small bacilli in the granular detritus that occupies the centre. Both clubs and bacilli are Gram-negative. A very pretty picture is obtained by staining with Ziehl-Neelsen and decolorizing cautiously with 1 per cent. H_2SO_4. The interior of the colony is blue, while the clubs, being weakly acid-fast, are stained red.

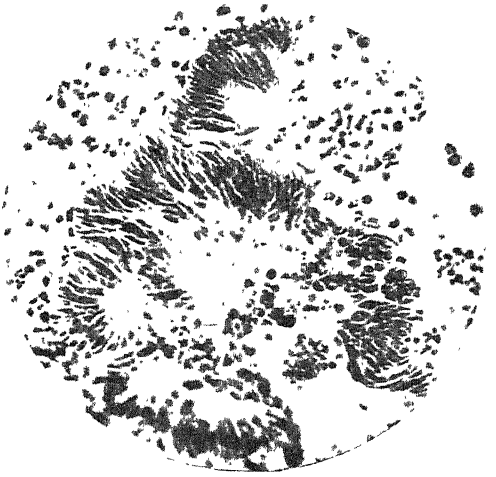


FIG. 270.

A colony of *Actinobacillus lignieresi* in the tongue of an ox, showing the long, finger-shaped clubs (× 350).

Cultures made from the granules revealed the presence of a very small

Gram-negative, non-motile, cocco-bacillus, which Lignières and Spitz called the actinobacillus—now called *Actinobacillus lignieresi.* (For description see Chapter 14.) In this country the disease is less acute, and the lesions are more localized, though the glands are almost invariably affected.

Reproduction of the Disease in Animals.—Subcutaneous inoculation of a pure culture into cattle leads to the formation of an abscess similar to that occurring in the natural disease, though not all strains are virulent. Fluctuation is apparent about the 10th day. The abscess enlarges, and eventually the skin gives way, allowing a small quantity of pus to exude. Fungating masses spring up around the opening and obstruct drainage. The animals gradually waste. The pus contains typical granules. Feeding has not been successful in transmitting the disease. A local abscess develops after subcutaneous inoculation of pure cultures into pigs and sheep. In horses a large œdematous swelling is formed, which discharges pus after a few days.

The work of Lignières and Spitz has been confirmed by workers in several countries. Nocard (1902) recognized the disease in France. He pointed out that in actinomycosis of cattle the granules in the pus are yellowish in colour and often calcified, whereas in actinobacillosis they are greyish-white and rarely calcified. Griffith (1916) examined the diseased tongues and lymphatic glands of 44 cattle slaughtered in this country. Of these no fewer than 40 showed lesions of actinobacillosis. From 23 of these he isolated a Gram-negative bacillus which had the characters of *Actinobacillus lignieresi.* One of the cultures inoculated subcutaneously into a calf caused a local lesion containing typical granules. The remaining 4 cases, affecting the lower jaw, were due to the *Actinomyces bovis.* In an examination of 34 specimens of " actinomycosis " in cattle slaughtered at Islington, Bosworth (1923) recognized *Actinobacillus lignieresi* 21 times; from 17 of the cases he isolated it in pure culture. The other 13 specimens were examples of actinomycosis.

Diagnosis.—The important points are: (1) The location: practically all jaw lesions appear to be due to *Actinomyces bovis,* while lesions of the soft parts—tongue, cheek, gum, palatal mucosa, skin, and lymphatic glands—are due to the actinobacillus. (2) The frequent involvement of the lymph glands in actinobacillosis; in actinomycosis the glands are swollen only as the result of secondary infection from open lesions. (3) Granules are rarely found in the glands in actinomycosis, very frequently in actinobacillosis. (4) In actinobacillosis the granules are paler in colour, and rarely calcified; in actinomycosis the granules are darker in colour and often calcified. (5) In actinobacillosis the granules consist of a central mass of detritus containing minute Gram-negative bacilli surrounded by long, radially disposed clubs; in actinomycosis recently formed granules contain a central Gram-positive mycelium, together with Gram-positive rods and coccoid forms, surrounded by short, radially disposed clubs; older granules may show a few Gram-positive bacillary and granular elements embedded in a Gram-negative matrix. (6) In actinobacillosis cultures reveal the presence of a small Gram-negative, aerobic cocco-bacillus; in actinomycosis a Gram-positive, branching, filamentous organism, often assuming rod forms in culture, not unlike *C. diphtheriæ,* is isolated under anaerobic conditions. During life the agglutination reaction may be of value. The serum of animals suffering from actinobacillosis frequently agglutinates the causative organism to a titre of 1/50 or over.

Actinobacillosis may affect *sheep*. According to Tunnicliff (1941), Christiansen in 1917 described a pyæmic infection of the heads of sheep due to an organism which he called *Bact. purifaciens*, but which was probably the actinobacillus. Thomas (1931), in South Africa, was the first to associate the disease in sheep with *Actinobacillus lignieresi*. The disease was relatively mild and occurred in animals fed on prickly pear. Lesions occur primarily in the lips, tongue, and cheeks, but the mandibular, retropharyngeal, mediastinal, and bronchial glands may also be affected. Single or multiple areas of granulation tissue are found on section, with burrowing irregular cavities containing sticky bright yellow pus, sometimes showing, on microscopical examination, ray-like formations and clubs. The disease in sheep has also been recorded by Ravaglia (1934) in Italy and Marsh and Wilkins (1939) in the United States.

Prophylactically, Lignières and Spitz (1902) found that segregation and killing of infected animals was the best procedure. Immune horse serum was employed in the treatment of some cases—though without conspicuous success. Iodide is said to be of much more value in the treatment of actinobacillosis than of actinomycosis.

So far as we know actinobacillosis is very rare in man. Ravaut and Pinoy (1911), Thompson and Willius (1932), Beaver and Thompson (1933), and Langeron (1941), have each described a case, but in no instance has the identification of the causative organism with *Actinobacillus lignieresi* been entirely satisfactory.

Pneumonia in Calves due to Actinobacillus actinoides.—Smith (1918, 1921) has described an epizootic broncho-pneumonia in calves, most common in the 2nd and 3rd months of life, characterized by areas of consolidation, focal necroses, and other lesions. From the lungs of a number of fatal cases in two separate outbreaks he isolated an organism which he called *B. actinoides* (see Chapter 11). In some cases it was present in pure culture, in others it was associated with *C. pyogenes*, *H. bronchisepticus*, or other organisms. On microscopical examination of the lungs, it was seen in the form of slender bacilli situated at the periphery of necrotic areas, aggregated into masses in the alveoli and alveolar ducts, or in the proliferating epithelium that occupied the smaller bronchioles. On coagulated horse serum, tiny whitish flocculi appeared in the water of condensation; these consisted of radiating filaments terminating in clubs. The organism was non-pathogenic to laboratory animals, but intratracheal injection into calves was sometimes followed by the appearance of small necrotic foci in the lungs, indistinguishable from those observed in the natural disease. This organism may be more closely related to *Actinomyces muris* (*Streptobacillus moniliformis*) than to the actinobacillus of Lignières and Spitz (see p. 381). Jones (1922) has isolated it from the pneumonic lungs of white rats.

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CHAPTER 55

SWINE ERYSIPELAS, ERYSIPELOID, MOUSE SEPTICÆMIA, AND INFECTIVE MONONUCLEOSIS OF RABBITS

SWINE ERYSIPELAS

Introductory

Swine erysipelas is a disease affecting mainly pigs, and caused by the slender Gram-positive bacillus, *Erysipelothrix rhusiopathiæ*. Adolescent animals seem to be most frequently affected, but it occurs at all ages except during the first 3 months of life, when it is uncommon. The finer breeds of pigs are particularly susceptible. Four clinical types of the disease are described (Craig 1926). (1) The acute or septicæmic form. After an incubation period of 1 to 5 days, illness commences with prostration and high fever, anorexia, thirst, occasional vomiting, and conjunctivitis. Twenty-four hours later bright or dark red patches appear on the skin over the ears, snout, neck, abdomen, and inner sides of the forelegs. The mortality is about 80 per cent., death occurring usually in 3 or 4 days. (2) The urticarial form, or "the diamonds." This is a mild form accompanied by slight malaise and fever, and characterized by the eruption on the 2nd or 3rd day of well-defined, quadrangular or rhombic patches on the skin of the sides, back, and buttocks; the patches are slightly swollen, deep red or violet in colour, sometimes with a pale centre, from 1–5 cm. in diameter, and may become covered with dry crusts which are afterwards cast off. Recovery occurs in a few days; death is unusual. (3) The chronic or cardiac form. This may follow either of the previous types, or may arise independently. Warty vegetations develop on one of the heart valves, generally the mitral. Death may occur suddenly, or the animals may live for several weeks with symptoms of cardiac insufficiency. (4) The joint or arthritic form. This may follow the urticarial form, accompany the cardiac form, or arise independently. The joints of the limbs become enlarged, due to a synovitis; the animals are stunted, prefer the recumbent posture, and when induced to get up, walk in a stiff fashion on their toes with the back arched. This form is not usually fatal, but interferes with growth and fattening.

Morbid Anatomy.—In animals dying of the septicæmic form the spleen is enlarged, the lymphatic glands are congested, the gastric and intestinal mucosæ are reddened, and the lungs are œdematous; there are sometimes small hæmorrhages on the serous and mucous membranes and beneath the endocardium. The bacilli are found in small numbers in the blood, spleen, kidneys, and other organs, and in the secretions and excretions. In the cardiac form they are chiefly found in, and may be confined to, the vegetations on the valves.

Epidemiology.—Swine erysipelas is common in this country and throughout Europe. In America it has apparently been uncommon in the past, but according

to Breed (1938) it seems now to be spreading and becoming of increasing importance. It occurs particularly in valleys and low-lying districts, and during the summer and autumn months. In this country, as a rule, only a few pigs in a herd are affected, but on the Continent large epidemics may occur. The causative bacillus has also been found to be responsible for outbreaks of polyarthritis in sheep, joint-ill in lambs, and for occasional infections in horses, cattle, turkeys, and peacocks (see Beaudette and Hudson 1936, Paterson and Heatley 1938, Greener 1939), and erysipeloid (see p. 1285) and endocarditis (Russell and Lamb 1940) in man.

The natural disease in pigs is apparently due to intestinal infection, though infection by the skin appears possible. The bacilli have been found in the gall-bladders of animals that have recovered from the mild type of the disease (Pitt 1908*b*), and from the tonsils and intestinal mucosa of normal swine (Pitt 1908*a*, Tenbroeck 1920), so that carriers may play an important part in the spread of infection. The bacilli are very resistant to the changes associated with putrefaction, and may remain alive for months in carcases of buried animals (Lösener 1896); it is possible, therefore, that infection may occur by food or water contaminated with infected soil.

The disease can be experimentally reproduced in swine by inoculation with pure cultures (see Chapter 15).

Diagnosis.—The bacteriological diagnosis of swine erysipelas is made by microscopic examination of the blood during life, and of the blood and viscera after death, for the characteristic slender Gram-positive bacilli. If the organisms are too few to be seen microscopically, they may be cultivated on agar, in broth, and in gelatin stabs. If contaminated or putrefying material alone is available for examination, then the mouse inoculation test should be performed (see p. 398), or the material may be plated on 5 per cent. blood agar at pH 6·8 containing 1/100,000 sodium azide and 1/500,000 crystal violet (Packer 1943). The agglutination test may be of value, particularly in animals showing evidence of joint involvement. The antigen should be prepared from a smooth strain (see Schoening and Creech 1936). During life examination of the blood reveals a leucopenia, an increase in the mononuclears, and the presence of many atypical large mononuclear and lymphocytoid cells (Egehoj 1938).

Prophylaxis and Serum Treatment.—General prophylactic methods are similar to those for anthrax (see Chapter 76).

The routine vaccination of herds has been attempted by two or three different methods (Voges and Schütz 1898). Pasteur and Thuillier (Pasteur 1882, Pasteur and Thuillier 1883) were responsible for the first method. They stated that if the virus was passed through rabbits, it gradually lost its virulence for pigs; on the other hand, if it was passed through pigeons, its virulence was so exalted that the final passage virus was more virulent for pigs than even the most infectious products of pigs dying naturally from swine erysipelas. Their first vaccine, therefore, consisted of the avirulent rabbit passage virus; their second, which was given about 12 days after the first, consisted of the hypervirulent pigeon passage virus. Though it is quite impossible from the available statistics (Chamberland 1894) to assess the value of this method, there is a general consensus of opinion that so long as its use is restricted to the more resistant commoner breeds of pigs and to areas in which the disease is rife, it results in a general lowering of the mortality from swine erysipelas. When, however, it is used for protection of the less

resistant finer breeds, it frequently causes severe losses, amounting to 5 or 10 per cent. of the animals inoculated. For this reason its use has been very largely abandoned.

The methods now employed are those of sero-vaccination. In Lorenz's method (1893, 1894, 1896), a dose of immune serum is injected, followed after about 4 and 14 days by injections of living virulent bacilli. The serum provides a passive immunity, which is converted by the living vaccines, given subsequently, into an active immunity. In Leclainche's method the living vaccine is mixed with the serum, and the two are injected together. A second injection of vaccine alone is given 10 or 12 days later. Both these methods have been widely used, and appear to give satisfactory results. Both of them are open to the objection that living bacilli are introduced into the body, with the result that the animals may become healthy carriers of the disease. Immunization by the use of dead bacilli has not yet proved practicable (see Watts 1940).

In the actual treatment of swine that have already developed the disease the injection of 10–30 ml. of immune serum is recommended.

ERYSIPELOID

Infection of man with the bacillus of swine erysipelas was first described by Rosenbach in 1884 (see Rosenbach 1909). Though, according to Verge (1933), a cutaneous, an intestinal, and a generalized form of the disease may occur, the first type, usually referred to as "erysipeloid," is undoubtedly the commonest. It is met with in cooks, kitchen workers, butchers, and in those who handle fish, game, or cheese (Klauder 1926, 1932, 1938). Epidemics of the disease have been reported. Stefansky and Grünfeld (1930) describe an outbreak at Odessa affecting about 200 persons engaged in handling freshwater fish brought by sailing ships from the Dnieper and Bug; while Lawson and Stinnett (1933) in the United States report 247 cases of erysipeloid occurring among workers employed in sawing and polishing bones in the manufacture of buttons.

Klauder (1932) says that infection can invariably be traced to contact with animals, fish, shell-fish, dead matter of plant or animal origin, or matter derived from animals such as hides, pelts, bones, and manure. The organism usually gains access through injuries on the skin, including the bites of animals, fish, and crustacea. Where the organism comes from, however, is not so clear. Direct transmission to man from swine suffering from erysipelas appears to be uncommon (Bierbaum and Gottron 1929), but it is sometimes met with in veterinary students (Morrill 1939). Circumstantial evidence suggests that the organism is present in the slimy coating of various fish, but, though its isolation from this source has been recorded by Schoop (1936), there is some doubt as to whether the slime is infected under natural conditions or whether it becomes contaminated from the flesh during preparation for market. Fish can readily be infected experimentally (Hettche 1937, Brunner 1938). The organism is known to be very resistant to salting and putrefaction, and to survive for a long time outside the body.

However it reaches the skin, it gives rise, after an incubation period of 1–4 days, to an erysipeloid lesion characterized by a sharply defined, slightly elevated, purplish-red zone, which extends peripherally at the same time as it fades and desquamates centrally. The rash is accompanied by considerable swelling, itching,

burning, and pain. Regional lymphangitis and lymphadenitis may develop. Arthritis of the finger joints is not unusual. Relapses are common, and second attacks may occur. The milder forms last 2–4 weeks, but the disease may continue for months. Death rarely occurs, though Russell and Lamb (1940) have described one fatal case of endocarditis in which the organisms were isolated from the blood six times during life and from the endocardial vegetations at autopsy (see also Klauder, Kramer and Nicholas 1943).

The bacilli may be cultivated from the deep layer of an excised piece of skin. Alternatively tissue fluid may be obtained for culture by injecting saline into the skin at the border of the lesion, and re-aspirating it without withdrawal of the needle.

According to Klauder (1938) the most satisfactory form of treatment consists in a wet dressing of 12 per cent. ichthamol in alcohol. Formerly he (1926) favoured serum treatment, but he has discontinued this owing to the frequency of serum sickness, which may prolong the period of incapacitation. He reserves serum treatment for infections persisting for a month or more, for rapidly progressing infections, and for cases complicated by arthritis. In these he injects 100–150 ml. during the course of a few days, most of it intramuscularly, but some around the local lesion. Judged by mouse experiments sulphanilamide and sulphapyridine do not appear to be of value (Porter and Hale 1939).

MOUSE SEPTICÆMIA

Infection with *Ery. muriseptica*—an organism closely related to, if not identical with, *Ery. rhusiopathiæ*—gives rise to a natural infection in mice, which spreads readily among a herd of these animals, sometimes assuming epidemic proportions. The disease was originally described by Koch (1880) as *mouse septicæmia*.

The experimental disease produced in mice by the inoculation of *Ery. muriseptica* corresponds exactly with that produced by *Ery. rhusiopathiæ* (see p. 398.)

INFECTIVE MONONUCLEOSIS OF RABBITS

In 1926 Murray, Webb and Swann at Cambridge described a natural disease of rabbits characterized by a large-mononuclear leucocytosis, and caused by a small Gram-positive bacillus to which they gave the non-committal name *Bact. monocytogenes*. This organism, which has now been met with in a wide variety of animals, is often referred to as *Listerella*, but we have given our reasons (p. 399) for believing that it is closely related to the swine erysipelas bacillus, and should be called *Erysipelothrix monocytogenes*.

Young rabbits, between 1 and 3 months old, are chiefly affected. They are undersized, usually develop a very distended belly, undergo progressive emaciation, and often die suddenly. At post mortem the subcutaneous tissue is found to be œdematous, looking like jelly. Clear serous fluid is abundant in the pericardium, pleura, and peritoneum. As a rule the lungs are œdematous, and contain one or more red infarcts, the result of infected emboli. The liver is congested and sometimes contains small, round, pale-yellow or grey areas of focal necrosis, 1·5 mm. or less in diameter. The spleen is usually small, shrunken, pale pink, and rather

tough; but in acute cases, and in cases in which there is extensive necrosis of the liver, the spleen is normal in size or slightly enlarged, soft, and of a dark purple colour. The adrenals are sometimes soft and diffluent. The mesenteric lymph nodes are always enlarged and œdematous. Culture of the organism is difficult; it may be successful from the mesenteric nodes; the heart's blood is usually sterile.

The disease can be reproduced experimentally, and is often characterized by a monocytosis, and focal necroses of the liver (p. 398).

DISEASES IN OTHER ANIMALS CAUSED BY ERYSIPELOTHRIX MONOCYTOGENES

Space does not permit a detailed description of the numerous types of disease to which this organism may give rise. Suffice it to say that it has been isolated by Pirie (1927) from a plague-like disease of gerbilles in South Africa, by Gill (1933, 1937) from an encephalitic disease of sheep in New Zealand known as "circling," by Jones and Little (1934) from cases of bovine encephalitis in New Jersey, by Tenbroeck (1932) from a sporadic disease of chickens in New Jersey, by Burn (1933–34, 1935, 1936) in the United States and by Gibson (1935) in Scotland from meningeal infections in human beings, by Seastone (1935) in the United States and by Paterson (1937) in England from a generalized infection of fowls, by Schmidt and Nyfeldt (1938) in Denmark and by Pons and Julianelle (1939) in the United States from cases of infective mononucleosis in man, by Biester and Schwarte (1939) from meningo-encephalitis of sheep in Iowa, by Biester and Schwarte (1940) from meningo-encephalitis of swine, and by Paterson (1940) in England from abortion in sheep. (For further references see Paterson 1939.)

The relationship of this organism to glandular fever is discussed on page 1791.

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CHAPTER 59

TUBERCULOSIS

In 1868 Villemin published his masterly study on the epidemiology of tuberculosis, and succeeded in transmitting the disease to animals by direct inoculation. Koch in 1882, after an investigation that will always remain as a classical example of thorough and accurate bacteriological technique, showed conclusively that the tubercle bacillus was the one essential cause of tuberculosis. The bacillus was resistant to all ordinary stains; but Koch succeeded in staining it by an alkaline solution of methylene blue, kept in contact with the tuberculous tissue for 24 hours. The bacillus would not grow on any ordinary medium; but he devised a new medium—inspissated blood serum—on which, after a delay of 10 days or so, growth first became apparent. Finally by a large series of inoculations with pure cultures of the bacillus, several generations removed from the primary one, he transmitted the disease to numerous animals of different species. Henceforward the demonstration of the bacillus afforded the sole infallible criterion for the diagnosis of all the manifold lesions of tuberculosis.

THE BACTERIOLOGY OF TUBERCULOSIS IN MAN

Tubercle bacilli may be divided by cultural and pathogenicity reactions into five types—human, bovine, murine, avian, and cold-blooded (see Chapter 16). Of these only the first two are found in natural infections of man. It is true that natural infections with the avian type have occasionally been described by continental workers, but the bacteriological evidence provided has not always been convincing. Feldman (1938), who reviewed the literature, could find a record of only 13 cases in which infection was probably due to the avian bacillus. In this country it was not found once in over 7,800 cases examined (Griffith 1938), but 2 cases have recently come to light.

The extensive work of the Royal Commission on Tuberculosis (Report 1911*a*), supplemented by the more recent findings of A. S. Griffith and of several other workers, have afforded us a knowledge of the frequency of tuberculosis in patients of different ages, which may be summarized in tabular form (Table 92). This table refers only to English cases. In Scotland the percentage of all forms of tuberculosis due to the bovine type is higher—often considerably so (see Griffith 1932*a*, 1934, Blacklock 1932, Griffith and Summers 1933, Blacklock and Griffin 1935, Munro and Scott 1936, Miller 1937, Griffith 1941). In Eire the information available suggests that the figures for non-pulmonary infections are much the same as in England (Mushatt 1940). Taking all ages into consideration, it will be seen that the majority of infections are due to the human type. About 1·5 per cent. of pulmonary and about 30 per cent. of non-pulmonary tuberculosis at all

ages in England and Wales appear to be caused by the bovine type. Infections of bovine origin are commonest during the first five years of life, and relatively uncommon in adults. The main organs affected by the bovine type are the gastro-intestinal tract and its associated lymphatic glands, particularly those of the cervical and mesenteric group. About 80 per cent. of cases of primary abdominal tuberculosis are due to the bovine type (Blacklock 1932, Griffith 1938); but secondary abdominal tuberculosis, which occurs most frequently as a late complication in pulmonary tuberculosis, is generally due to the human type. Lupus

TABLE 92

FREQUENCY OF BOVINE TUBERCLE BACILLI IN HUMAN BEINGS. ENGLISH STATISTICS. (Griffith 1938.)

Variety of Tuberculosis.	0 to 4 years.	5 to 14 years.	All ages.	
	Per cent. Bovine	Per cent. Bovine.	Number of Cases.	Per cent. Bovine.
Cervical gland	91·3	54·2	128	50·0
Lupus	58·4	44·4	191	48·7
Scrofulodermia	53·3	43·3	60	36·6
Bone and joint	29·5	19·1	554	19·7
Genito-urinary	—	—	23	17·4
Meningeal	28·1	24·5	265	24·6
Pulmonary	—	—	3,103	1·4
Miscellaneous	33·3	9·1	23	8·7

occupies a peculiar position; 48·7 per cent. of the strains are of bovine and 51·3 per cent. of human type. But generally both human and bovine strains are atypical; their cultural reactions conform to the standard of one or other type, but their virulence for animals is lowered. Since these atypical variants are found almost exclusively in lupus, it is probable that the degradation in virulence results from the sojourn of the organisms in the skin (Report 1911*b*). There is no evidence that it is due to the effect of light, as has been supposed, since organisms of lowered virulence are isolated both from exposed and unexposed areas. A. S. Griffith (1924) concludes that the changes occurring in the organisms cultivated from lupus lesions are not merely degenerative, but are the result of adaptation of the bacilli to sojourn in the skin. As a rule, by passage of atypical bovine strains through calves or rabbits, their full virulence is regained. Passage of atypical human strains through guinea-pigs or monkeys has not been successful in restoring their virulence (A. S. Griffith 1924). Atypical strains may be found in conditions other than lupus; thus in an investigation of 261 strains isolated from human cases of bone and joint tuberculosis Eastwood and F. Griffith (1916) encountered 10 atypical strains. Sometimes these atypical strains are found on further investigation to consist of a mixture of bovine and human bacilli (Report 1911*c*).

Approximately one-quarter of all cases of tuberculous meningitis and cerebral tuberculosis appear to be due to the bovine type (see Griffith 1932*b*, 1934, Blacklock and Griffin 1935, Griffith and Menton 1936). The proportion is greater in the lower than in the higher age groups.

Pulmonary tuberculosis has been generally held in the past to be due almost exclusively to the human type of bacillus. A. S. Griffith (1914), for example,

collected figures referring to 938 cases in which the causative organism had been typed, and found that in only six had a bovine type been isolated: in three of these it had been associated with a bacillus of human type. More recently, however, this conception has been challenged (see Munro and Griffith 1928, Griffith 1930, 1940, Griffith and Munro 1933, Cumming *et al.* 1933, Walker 1934, Griffith and Smith 1935, 1938, 1940, Tobiesen *et al.* 1935*a*, *b*, Lange, B. 1937). It has been shown that in Scotland and in Northern England an appreciable proportion of cases are due to infection with the bovine type (Table 93). The reason for the differences between different parts of the country is to be found in the higher incidence of tuberculosis in cattle in the northern half of Great Britain.

TABLE 93

FREQUENCY OF BOVINE TUBERCLE BACILLI IN PULMONARY TUBERCULOSIS IN DIFFERENT PARTS OF GREAT BRITAIN AND IRELAND.

(Cumming 1935, Griffith and Smith 1940, Griffith 1941, Griffith and Munro 1944, Cuthill and Lynn 1944.)

Area	No. of Strains Examined	Percentage Bovine.
Scotland, North-east	972	7·0
Rest of Scotland	1,797	5·1
England, North and Middle	3,219	2·0
Wales	201	1·0
England, South	989	0·6
Ireland	320	0·0

Summarizing, we may say that infections in this country with bovine tubercle bacilli occur mainly in children; in adults they are less common. The usual types of infection due to the bovine bacillus are glandular and alimentary tuberculosis, together with miliary tuberculosis and meningitis in infants and young children. In pulmonary tuberculosis, though it is very much less common than the human bacillus, it is sufficiently frequent to demand serious attention.

EPIDEMIOLOGY

Tuberculosis is an infectious disease that spares neither age, sex, race, nor nationality. The pulmonary form was described by Hippocrates, and spinal caries has been observed in the mummies of ancient Egypt (Smith, E., 1909). With the spread of civilization it became more rife; but now, in the more highly civilized countries of the world, it is declining. The main features of its epidemiology, as enumerated by Villemin (1868), are still generally true:

(1) Tuberculosis is uncommon in mountainous areas. It is comparatively rare in the mountains of Hungary and Styria, in the Carpathians, the Engadine, the Pyrenees, the high plateaux of Abyssinia and Armenia, in the Western Ghats and the Nilghiries, in the Himalayas, the Rockies, the Andes, and the high plateau of Mexico.

(2) It increases proportionately to the degree of crowding, and is especially common in the capitals, and great commercial and manufacturing towns.

(3) It attacks particularly persons who live in closed communities, such as prisoners, soldiers, and religieux.

(4) It spares those people who live in small communities, in the open air, or

in a savage or nomadic state. The wandering Arabs, for example, rarely contract tuberculosis; but it is common amongst the Arabs who have exchanged their tents for stone houses.

(5) It is very common among troops living in barracks, but is uncommon in the field and among troops who are not living in barracks. At the time at which Villemin wrote, the mortality from tuberculosis in the Army during peace time was from 5 to 12·5 per 1,000; in the field, however, the mortality was very much lower. As an example, he quotes the case of the Allied Armies before Sebastopol during the winter of 1854–5; even though they were living under most unhygienic conditions, and were subject to poor food, severe cold, and excessive fatigue, cases of tuberculosis were very rare

(6) Close contact in small, badly ventilated dwellings leads to the development of the disease.

(7) Though at one time unknown in Australia, New Zealand, North America, and parts of Polynesia, it has become a scourge in these countries since the advent of Europeans.

(8) Bovine tuberculosis, like human, increases with close contact and overcrowding; when it appears in a byre it generally attacks large numbers of animals.

All these facts bring tuberculosis into line with the other zymotic diseases.

Mortality from Tuberculosis.—Table 94 shows the annual mortality from tuberculosis of all forms and of the respiratory system in this country during the last 80 years.

TABLE 94

SHOWING THE ANNUAL MORTALITY PER 1,000 PERSONS LIVING IN ENGLAND AND WALES FROM TUBERCULOSIS. (STANDARDIZED DEATH RATES.)

Years.	Tuberculosis (all forms).	Tuberculosis of Respiratory System.
1851–55	3·638	2·890
1856–60	3·328	2·663
1861–65	3·316	2·625
1866–70	3·217	2·558
1871–75	2·956	2·327
1876–80	2·818	2·141
1881–85	2·558	1·922
1886–90	2·342	1·704
1891–95	2·138	1·504
1896–1900	1·912	1·337
1901–05	1·739	1·208
1906–10	1·556	1·082
[1] 1911–15	1·389	1·005
[1] 1916–20	1·359	1·009
1921–25	1·065	0·816
1926–30	0·922	0·721
1931–35	0·782	0·620
1936	0·658	0·526
1937	0·658	0·524
1938	0·603	0·477

[1] Mortality refers to civilians only.

It will be seen that the mortality has steadily declined since 1851—the first year for which standardized death rates are recorded. Several causes have been alleged for this decrease, such as better housing and sanitary conditions, improved

social habits, diminished drunkenness, higher standard of living, earlier diagnosis, segregation of advanced cases, sanatorium treatment, and the development of a racial immunity. It is extremely difficult to assess the importance of these factors. Greenwood and Wolff (1928) are of the opinion that greater weight must be ascribed to social, hygienic, and cultural improvements than to biological factors, such as the progressive increase in the resistance of the race through natural selection and acquired immunity.

Wolff (1935) points out that the tuberculosis mortality has declined more in the industrialized States of Europe than in those which are still mainly agricultural. The reason for this, he believes, is that the standard of living and general social hygiene are on a higher level in the industrially developed than in agricultural countries. Much the same thesis is put forward by Baskett (1931), who maintains that in all civilized countries the phthisis mortality is inversely correlated with the level of real wages. That biological factors are also operating, however, is almost certain. Ostenfeld, Heitmann, and Norlyk (1931), for example, point out that in Sweden during the past century there has been a centrifugal spread in the frequency of the disease. A hundred years ago the highest death rate from tuberculosis was in Sodermanland, where now it is lowest; while Norrbotten, which 100 years ago was relatively free from the disease, is now specially affected. The suggestion is that the resistance of a population in which the disease has been prevalent for some generations is greater than that of a population coming into contact with tuberculosis for the first time. Further evidence in support of this thesis will be found in a later section dealing with tuberculosis in native races.

Among the social factors that are of importance great stress is laid by Wolff (1936*b*) on diet and by Hart and Wright (1939) on housing. There is little exact knowledge on the influence of *diet*. Shortage of first-class proteins was regarded by Faber (1938) as being largely responsible for the rise in tuberculosis mortality that occurred in western Europe during the war of 1914–18. Experimentally there is some evidence that animals fed on a plentiful diet are more resistant to infection than those on a meagre diet, and that Vitamin A deficiency may lower their resistance (see Robertson 1934, Clausen 1934, and Chapter 54). Kon and Maddock (1938), however, were unable to confirm the importance of Vitamin A in the protection of pigs against experimental infection. Osborn and Gear (1940) have pointed out that animals, like the dog and the rat, which can synthesize their own Vitamin C, are more resistant than those, like man, the monkey, and the guinea-pig, which cannot. During the war of 1914–18 the death rate from tuberculosis in German cities, which was only 1·57 per 1,000 in 1913, rose in 1918 to 2·87. In Warsaw by 1917 it had reached 8·4 per 1,000, and in Belgrade, as the result of the military occupation and want of food, it rose to the enormous figure of 14·0 per 1,000 (Dublin, see Opie 1929). In the second world war there was again an increase in mortality. The factors that may have played a part in this rise in Great Britain are discussed in a report by the Medical Research Council (Report 1942).

Without in any way minimizing the desirability of active public measures against tuberculosis, it may be pointed out that the decline in the tuberculosis death rate in this country set in long before the establishment of hospitals, sanatoria, or dispensaries for the treatment of patients suffering from the disease, indicating that the underlying social, economic, and biological causes were able by themselves to effect a considerable reduction in the mortality from tuberculosis in the absence of any *ad hoc* public health interference.

Some Factors affecting the Mortality from Tuberculosis.

Occupation.—Occupation is of importance. In this country the mortality from pulmonary tuberculosis is below the average for all males in fishermen and coal-

miners, but is high in stone and slate quarrymen, in cutlers, file-makers, earthenware manufacturers, and highest of all in the Cornish tin-miners. All trades, in fact, in which the men are exposed to the inhalation of particles of metallic or stone dust, particularly dust containing silica, have a high mortality rate from pulmonary tuberculosis. Both experimental and field work have shown the predisposing action of silica towards tuberculosis (Gye and Kettle 1922, Kettle 1924, 1930, Hart and Aslett 1942).

Though not strictly relevant to occupation, it may be pointed out that tuberculosis mortality is much higher in *mental* patients, particularly in schizophrenics, than in the general population. For example, according to Deegan, Culp and Beck (1942), the death rate from tuberculosis during 1937–39 in mental institutions in New York State, outside New York City, was 6·18 per 1,000 as against 0·51 per 1,000 in the population as a whole, that is, over 12 times as high.

Age and Sex.—We have no space to analyse the age and sex incidence fully. Table 95 shows the high mortality of the disease in the 0–5 age group; the fall during the period of adolescence; the great increase in mortality, especially in women between the ages of 15 and 30; and the high mortality in men between the ages of 40 and 55 from what is known as the late adult type of tuberculosis.

TABLE 95

DEATHS FROM TUBERCULOSIS IN 1934 IN ENGLAND AND WALES CLASSIFIED ACCORDING TO AGE AND SEX.

Age Group.	Tuberculosis (all forms).		Tuberculosis of Respiratory System.	
	Males.	Females.	Males.	Females.
All ages	17,448	13,434	14,734	10,948
0–1	242	171	39	18
1–5	693	615	83	65
5–10	337	349	69	59
10–15	335	414	101	214
15–20	901	1,412	688	1,198
20–25	1,843	2,194	1,604	1,983
25–30	1,748	1,905	1,570	1,757
30–35	1,678	1,485	1,527	1,369
35–40	1,494	1,118	1,371	1,022
40–45	1,473	900	1,378	796
45–50	1,707	776	1,624	705
50–55	1,662	624	1,576	546
55–60	1,402	510	1,316	435
60–65	927	382	889	333
65–70	637	289	580	229
70–75	232	168	209	131
75–80	102	80	85	63
80 and upwards	35	42	25	25

During the present century a great change has come over the age and sex distribution of tuberculosis. Reference to Fig. 271 will show that during last century and the first decade of the present century the mean annual death rate per 100,000 living was highest in infancy and early childhood, while in 1933 it was highest in the 20–25 age group.

The fall in the death rate during the early years of life has been most striking. This is almost certainly attributable to some extent to the much greater care now

exercised in infant welfare, and to the more general provision of pasteurized milk (see Drolet 1930). It may be noted that the highest tuberculosis mortality in children occurs during the 1–2 age group, and is due to various types—respiratory, meningeal, abdominal, miliary, and chronic disseminated. Meningitis alone is responsible for about 50 per cent. of the total deaths during the first 10 years of life.

The death rate in young adults, on the other hand, has undergone much less alteration. Most of the deaths in this group are due to pulmonary tuberculosis,

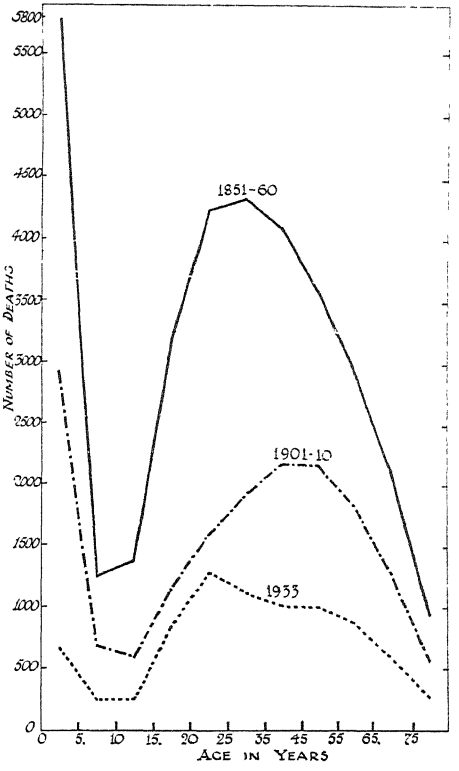


FIG. 271.

Death rates per million from tuberculosis (all forms) at ages. England and Wales 1851–60, 1901–10, and 1933.

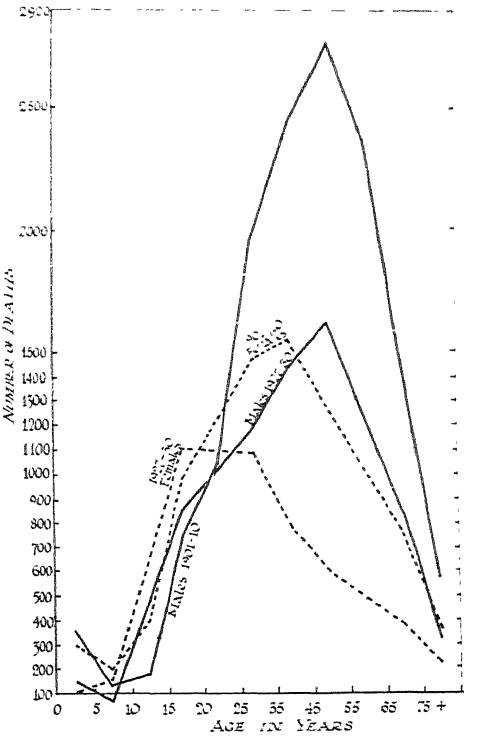


FIG. 272.

Death rates per million from pulmonary tuberculosis at ages for each sex. England and Wales 1901–10 and 1927–30.

and as Fig. 272 shows, the death rate from this form is much higher in females than in males in the 10–30 age group. Indeed the female pulmonary tuberculosis death rate in the 20–25 age group is practically identical with that in the first years of the century.

After the age of 30 the male pulmonary tuberculosis death rate increases, till in the 45–55 age group it is nearly three times that of the female death rate. The reasons for these differences are not apparent, and though they are often lightly attributed to changes in the general mode of life of young women during the present century, and to the greater strain of competitive existence among men, the reasons

are undoubtedly complex, and we shall do well to refrain from attempting to simplify them unduly. We may note that the changes in the age and sex distribution of tuberculosis are not confined to this country, but are reflected in greater or less degree in the statistics of many other European countries and of the United States of America (see Dow and Lloyd 1930, Drolet 1930, Whitney 1931, MacNalty 1932, Hill 1936, Hart and Wright 1939).

Race and Civilization.—Certain races seem to be more susceptible than others; thus the Negroes in North America suffer much more severely than the white population. In eight of the great cities of the United States, the mortality from tuberculosis in 1920 amongst the white population varied from 0·794 to 1·216 per 1,000

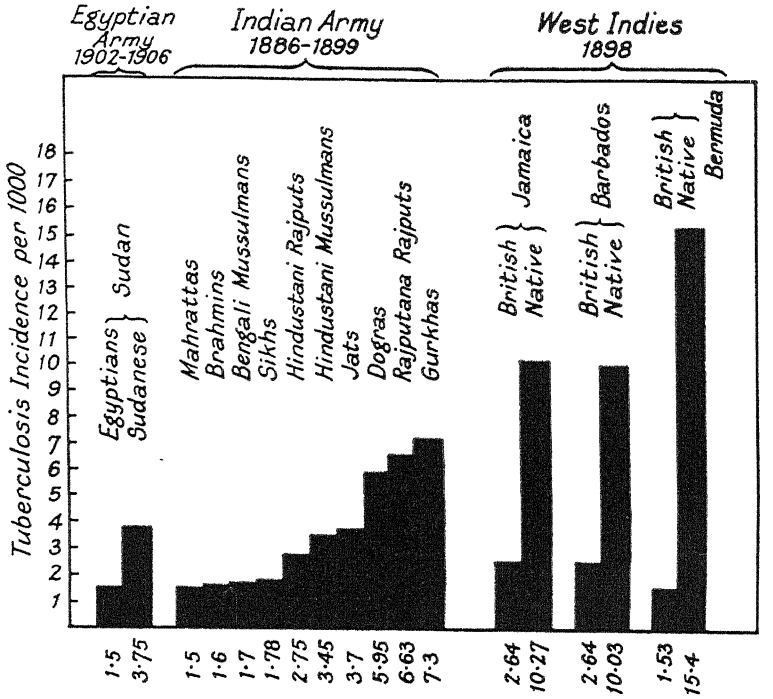


FIG. 273.
(After Cummins.)

living, whereas amongst the coloured population it varied from 2·855 to 4·405 per 1,000 (Mossell 1923). Jews, on the other hand, are said to have a high resistance (see Arnould 1936). In Fig. 273 is represented the incidence per 1,000 from tuberculosis amongst different races living under military conditions.

It will be seen that the differences in mortality between different races are very striking. There are so many factors influencing the distribution and mortality of tuberculosis that it would be unjustifiable to conclude that all these differences are due to a lower *genetic* resistance of the coloured than of the white races. The environment in which the two races live, the level of personal and social hygiene, the nature of the diet, and numerous other factors vary widely, and we know of no data in which the mortality of the disease is compared in coloured and

white subjects living under identical conditions and exposed to the same risk of infection from birth upwards (see Krause 1928, Johnson and Myers 1933, Scott 1935).

In primitive peoples living in their native countries tuberculosis either does not occur or is comparatively uncommon. This freedom from tuberculosis appears to be not an expression of the inherent resistance of man living under natural conditions, but is rather due to the lack of opportunity of infection with the tubercle bacillus. Once this organism is imported—generally by the advent of white Europeans—the disease may spread rapidly, provided the environmental conditions are suitable. The same happens when primitive peoples leave their native country and take up residence in a civilized area. Many of them become infected, contract a severe form of the disease, and die.

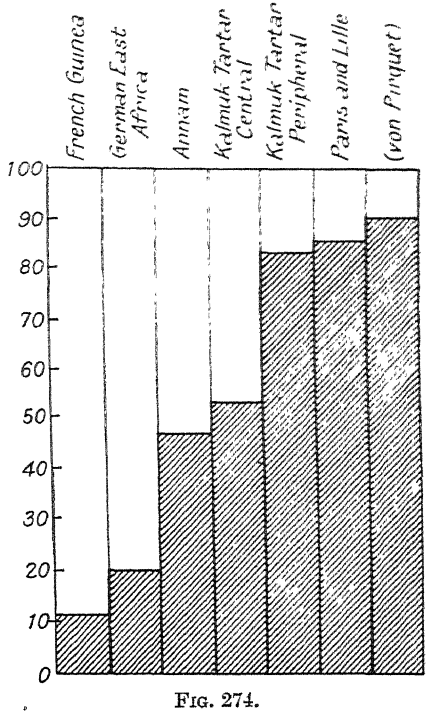


FIG. 274.

Comparative percentages of positive cutaneous tuberculin reactions in primitive and civilized peoples. (After Cummins.)

This sequence of events has been noticed by several competent observers. Cummins (1908) found that the death rate from tuberculosis amongst the Sudanese in the Egyptian Army was considerably higher than amongst the Egyptians (Fig. 273). In their native country tuberculosis was rare; but when they were brought into contact with more civilized peoples, they proved to be very susceptible to the disease. Metchnikoff, Burnet, and Tarassevitch in 1911 reported that the nomadic Tartar Kalmuks 50 years ago were practically free from tuberculosis; but that under the influence of increasing contact with the Russians, they were becoming more and more infected by the disease. In the central parts of the Steppes cases were uncommon, but at the periphery, where contact with civilization occurred most freely, tuberculosis was very frequent. Coleman, reporting on the North American Indians, brought evidence to show that tuberculosis was unknown before the advent of Europeans. With the advance of civilization, however, it has become increasingly common. The toll of life levied by the disease in some tribes is enormous; in 1896, for example, amongst the Oglalas the incidence of tuberculosis was 148·7 per 1,000, and the death rate 24·88 per 1,000. Borrel's (1920) observations on the Senegalese troops during the war of 1914–18 are of interest. In the villages and interior of Senegal tuberculosis is rare; but when the Senegalese troops were brought to France, large numbers of them developed tuberculosis. The longer their stay was in France, the higher was the incidence of tuberculosis amongst them. After 1 year's stay the mortality from tuberculosis was 2 per 1,000; after 3 to 4 years' stay it was 16 per 1,000. In 1918 the case incidence was as high as 36·1 per 1,000. (For references to these and other observations see Cummins 1911–12, Westernhoeffer 1911, Bushnell 1920.) A study of the tuberculin reaction (see p. 1300) shows that in primitive peoples the proportion of positive reactors is low; but that the more they come into contact with civilization, the greater is the proportion of reactors (Fig. 274).

Not only, however, is the mortality from tuberculosis high amongst primitive peoples brought into contact with civilization, but the type of disease tends to differ from that usually met with in civilized countries. Instead of the localized, slowly spreading, ulcerative type of phthisis, the disease often pursues a rapid course, fatal in a few weeks to a few months. Pathologically, the glands at the site of infection—generally the tracheo-bronchial group—are enormously enlarged and caseating, and there are miliary tubercles throughout the body. The tissues seem to be devoid of resisting power; there is no fibrosis, no limitation of the lesions, and no attempt at healing.

This acute type of tuberculosis recalls the type that is met with in infants of civilized peoples. A fairly high proportion of infants who become infected with the tubercle bacillus develop an acute, fatal disease, generally of the miliary type. That this is true is shown by the combined evidence of the tuberculin test, the mortality statistics, and post-mortem findings (see p. 1303). After the first year of life in civilized peoples, tuberculosis assumes a subacute or chronic course, and the miliary spread is seen only as a fatal termination, not as a primary manifestation of the disease. But in uncivilized races brought for the first time into contact with tuberculosis, the acute, fatal, miliary type is seen quite commonly even in adults.

At the present time there must be very few native races left that have not had some degree of contact with civilization. Tuberculin surveys carried out in recent years have shown that most native races are now undergoing a process of tuberculization, and it is to be expected that the acute miliary type so often met with in peoples brought into contact with tuberculosis for the first time will gradually give way to the more chronic types characteristic of civilized peoples. See Paneth (1928), Scott (1929, 1935), Editorial (1929), Duchêne (1929), Goodale and Krischner (1930), Kleine (1930), Schwetz (1930), Schwetz *et al.* (1930), Grasset and Perret-Gentil (1930), Whitney (1931), Pinner and Kasper (1932), Report (1932*b*), Review (1933), Sauvan (1935), Rodríguez Pastor *et al.* (1935), Long (1935), Cochrane (1937), Carmichael (1938), and numerous papers in the *American Review of Tuberculosis* and the *Tropical Diseases Bulletin* during 1936 to 1945.

Heredity.

The part played by heredity in predisposing to tuberculous disease is still an open question. Before the discovery of the tubercle bacillus, the disease was believed to be inherited. It is difficult to decide, however, from the observation that the disease tends to run in families, how much is due to inferior genetic resistance and how much to household exposure to infection. The milder nature of the disease in old-established civilizations than in primitive peoples coming into contact with civilization for the first time may be due to a process of weeding out by natural selection of the less genetically resistant members of the population. Explanations of this sort, however, though plausible, cannot carry conviction. As has already been pointed out (p. 1296) only a comparison of the morbidity and mortality of the disease in, for example, coloured and white subjects living under identical conditions and exposed to the same risk of infection from birth upwards can give us a satisfactory answer. Recent observations on animals and on human beings, however, do suggest fairly strongly that inherited resistance to tuberculosis varies widely.

Carmichael (1938), for example, found that the incidence of tuberculosis among the long-horned Hamitic cattle belonging to the Bahima people of Ankole was much higher

than among the humped Zebu cattle of Uganda, even though the environmental conditions appeared to favour the Hamitic variety. Though these animals lived almost entirely in the open air, 80 per cent. of them reacted to tuberculin, as against less than 1 per cent. among the Zebu cattle. Besides being resistant to natural infection, the Zebu cattle proved almost entirely refractory to experimental inoculation. Lurie (1941) studied six inbred families of rabbits, and found a wide difference in their response to infection with bovine tubercle bacilli, whether inhaled as the result of natural contact or inoculated parenterally. In the animals of the most resistant family the lung disease tended to be localized, and the primary focus progressed slowly, became encapsulated, and underwent cavitation. In the animals of the most susceptible family little or no localization occurred, and the disease was of the rapidly progressive generalized type. It is important to point out that in all the animals the disease was progressive; the chief difference lay in the rate of progression. It was also found that when the bacilli were administered artificially by the respiratory tract, the difference between the families was noticeable only when the dose was small; above a critical level the rabbits of even the most resistant family succumbed rapidly to the infection. No one factor seemed to be responsible for the differing resistance of the different rabbit families. Several factors were apparently implicated. Thus it was found that low skin permeability to the injection of Indian ink, rapid and intense development of allergic sensitivity, and rapid and high production of antibodies were associated with resistance; whereas high skin permeability, slow and feeble development of allergic sensitivity, and tardy and poor production of antibodies were associated with susceptibility (see also Lurie 1944*b*).

A careful study by the twin family method on the susceptibility of human beings to tuberculosis by Kallmann and Reisner (1943*a*, *b*) goes far towards confirming the conclusions reached from animal experiments. In co-operation with the tuberculosis hospitals and clinics in the State and City of New York a study was carried out on 308 complete twin families, comprising 616 twin partners, 930 full siblings (both parents in common), 74 half-siblings (one parent in common), 688 parents and 226 marriage partners of twin patients. Among the 308 twin pairs there were 78 monozygotic (uniovular) and 230 dizygotic (binovular) pairs. When a twin developed tuberculosis in an institution or was admitted to a hospital or clinic, the other twin was examined. The first twin was termed the index case. The investigation was restricted to pulmonary tuberculosis in twins 15 years of age or over. Corrected tuberculosis morbidity expectation rates were worked out to estimate the chances of a relation of a twin index case developing manifest tuberculosis; they were as follows: Marriage partners 7·1 per cent.; half-siblings 11·9 per cent; parents 16·9 per cent.; full siblings 25·5 per cent.; dizygotic twins 25·6 per cent.; monozygotic twins 87·3 per cent. These figures indicate that the chances of developing tuberculosis increased in proportion to the degree of blood relationship to a tuberculous index case. Monozygotic twin partners, it will be noted, were 3·5 times as likely as dizygotic twin partners to develop tuberculosis if the co-twin had tuberculosis. These differences could not be explained on the basis of greater exposure of monozygotic twins to infection. It was further observed that the morbidity expectation among siblings and dizygotic twins was about twice as high for the descendants of tuberculous parents as for descendants of non-tuberculous parents. Further studies along these lines in other parts of the world will be awaited with interest.

The Frequency of Tuberculosis.

In this country tuberculosis is a notifiable disease, and it might be thought that the number of notified cases would afford a fair estimate of the frequency of the

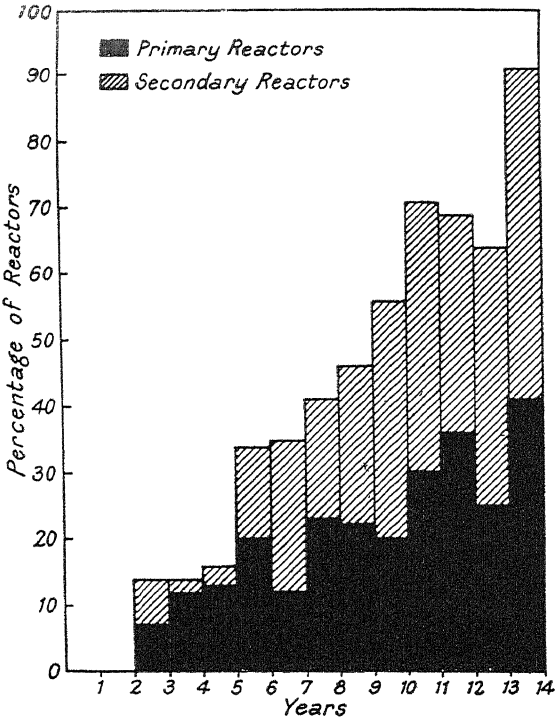


FIG. 275.

(Modified from von Pirquet.)

disease. In fact, however, this is not so. For various reasons —medical and social—large numbers of cases are not notified, and the resulting figures sometimes scarcely exceed the deaths. They are in fact so unreliable that, since 1931, the Registrar-General has ceased to publish them in his Annual Report. Neither the morbidity nor the mortality statistics afford any adequate idea of the prevalence of tuberculous infection. To obtain a satisfactory idea of this, it is necessary to resort to three other sources of information, namely, the tuberculin test, post-mortem statistics, and X-ray examination.

Latent Tuberculosis as revealed by the Tuberculin Test. —The tuberculin test as used by von Pirquet (1907) consists in placing a drop of tuberculin on the forearm and a drop of 50 per cent. glycerine adjacent to it as a control. With a small lancet a scratch is made through each of the drops. A positive reaction is denoted by the appearance within 24 or 48 hours of a bright red papule, at least 5 mm. in diameter at the site of inoculation of the tuberculin; later the colour becomes dark red, and the lesion disappears in about a week. This may be called a primary positive reaction. In some persons, however, the reaction is delayed for a few days, or occurs only after a second inoculation a week later—torpid or secondary reaction. From a large series of tests, it would appear that a primary positive reaction is evidence of the existence in the body of a comparatively recent tuberculous lesion, which may or may not be actively progressing. A torpid or secondary reaction is believed to indicate the existence of an old tuberculous lesion in the process of healing. Complete failure to react is regarded as evidence that the person has never come into contact with tuberculosis, or that healing has occurred. Von Pirquet's original technique has been modified by many subsequent observers, some of whom lay stress on the advantages to be gained by using varying dilutions of tuberculin, and so obtaining quantitative results.

Using this test, von Pirquet (1909) examined 1,134 clinically non-tuberculous infants and children in the Escherich Clinic at Vienna. The patients belonged to the lower classes, and were probably more heavily infected with tuberculosis than were the children of the higher social classes. It is important to remember in interpreting the results, given in Table 96 and Fig. 275, that tuberculosis was

at the time of the investigation exceedingly rife in Vienna; it is not justifiable, therefore, to regard them as generally applicable to every urban population.

TABLE 96

SHOWING THE PERCENTAGE OF CHILDREN WHO REACTED TO THE TUBERCULIN TEST IN VIENNA. (Modified from von Pirquet.)

Age	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14
No. of cases	388	89	75	87	62	66	61	39	72	45	44	45	39	22
Primary positive	0	0	7%	12%	13%	20%	12%	23%	22%	29%	30%	36%	25%	50%
Torpid or secondary positive	0	0	7%	2%	3%	14%	23%	18%	24%	36%	41%	33%	39%	41%
Total reactors	0	0	14%	14%	16%	34%	35	41%	46%	65%	71%	69%	64%	91%

The figures for the older age groups are rather small; but it is clear that by the age of 10 years about 70 per cent., and by the age of 14 probably over 90 per cent., of children were infected with tuberculosis. It will be noticed that torpid or secondary reactions were uncommon under 5 years, but gradually increased with age; this may be taken to indicate that quite a high proportion of children between 5 and 14 had old, and probably healing, tuberculous foci.

Similar investigations undertaken by various workers have shown that in the large cities of Europe, such as Paris, Vienna, and Prague, about 20 per cent. of children are infected at the age of 2, 55 per cent. at 5, and 90 per cent. at 15; of adults 97 per cent. are infected (Mantoux 1910, Calmette *et al.* 1911).

The von Pirquet test is now being gradually displaced by the *graded intradermal* or *Mantoux test.* In surveying a given population it is usual to inoculate 0·1 ml. of a 1/10,000 dilution of old tuberculin (O.T.) intradermally into the forearm. A positive reaction is characterized by the development within 12–72 hours of an area of œdema or induration at least 5 mm. in diameter; many workers insist on a minimal diameter of 10 mm. Erythema of varying extent is usually present, but by itself does not constitute a positive reaction. If the reaction is negative after 48 hours, a second injection is given, using 0·1 ml. of a 1/100 dilution. Lower dilutions are now seldom employed, since the reactions to them are often non-specific. In the past an injection with a 1/1,000 dilution was commonly used for negative reactors to the 1/10,000 dilution; but experience has shown that it is safe to proceed directly to a dilution of 1/100. A positive von Pirquet test corresponds approximately to a reaction to the intradermal inoculation of 1/1,000 tuberculin. Since the use of a 1/100 intradermally may detect 5–15 per cent. more reactors than the 1/1,000 dilution (Hetherington *et al.* 1929, Dow and Lloyd 1931, Hart 1932), it is clear that the graded intradermal test is more sensitive than the von Pirquet test. Old tuberculin is now often replaced by the purified protein derivative of tuberculin (P.P.D.) described by Seibert (1934, 1941*a*). For routine purposes this is given usually in a first dose of 0·00002 mgm. and a second dose of 0·005 mgm., corresponding approximately to doses of 0·002 mgm. and 0·5 mgm. of O.T. (Long, Aronson and Seibert 1934). There is reason to believe, however, that the second dose is too strong and may give a certain proportion of false positive reactions (Long and Seibert 1937, Furcolow *et al.* 1941). The two products generally give similar reactions in survey work (see Plunkett and Siegal 1937, Doig *et al.* 1938); but sometimes there is a disagreement which, according to Seibert and Du Four (1940), may be due to the presence in O.T. of non-specific substances that augment the specific reaction (see also Vollmer and Ripps 1943). Though in this country it is usual to refer to the actual dilution injected, in the United States the dose is expressed

in milligrams of tuberculin; by this notation 1 mgm. of tuberculin is supposed to be contained in 0·1 ml. of a 1/100 dilution.

Some of the results obtained during recent years by the graded intradermal test are reproduced in graphical form in Fig. 276.

It is apparent that in the large industrial cities of Europe and America most children are infected with tuberculosis by the time they reach 18 years of age, but in rural areas infection is much less frequent. Since only a comparatively small proportion of reacting children develop clinical tuberculosis or die of the disease, it is clear that in the majority of them the infection must remain latent. This deduction is of fundamental importance to a proper understanding of the mechanism of immunity in tuberculosis.

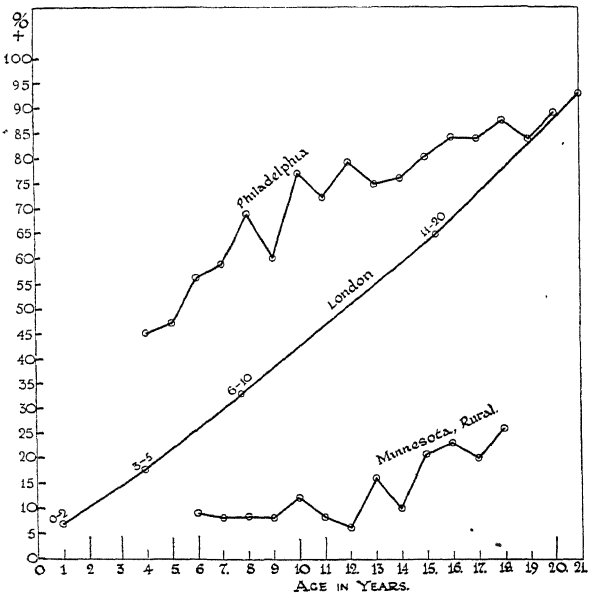


Fig. 276.

Percentage of children and adolescents reacting to the intradermal inoculation of old tuberculin.
(1) Philadelphia school children reacting to 1/100 dilution (Hetherington *et al.* 1934).
(2) London hospital children reacting to 1/100 dilution (Hart 1932).
(3) School children in wealthy rural community in Minnesota reacting to 1/1000 dilution or to von Pirquet test (Slater and Jordan 1932).

It has been shown by a number of workers that children brought up in contact with open cases of tuberculosis react to tuberculin at a much earlier age than normal children. Fig. 277, taken from Dow and Lloyd (1931), shows that over 70 per cent. of children of such parents react positively by the age of 5 years. In children brought up in contact with tuberculous parents having a negative sputum the proportion of tuberculin-positives is little higher than in normal children.

Further references to tuberculin surveys will be found in papers by Scheel (1929) Myers (1930), Myers *et al.* (1934), Brink *et al.* (1933), Cummins and Evans (1933), Drolet (1934), van den Berg (1936), and in numerous papers in the *American Review of Tuberculosis*, the *American Journal of Public Health*, and the *Bulletin of Hygiene* during the last few years.

Latent Tuberculosis as revealed by Post-mortem Statistics.—It is one of the commonest experiences of the post-mortem room to find evidence of tuberculosis in persons who presented no clinical evidence of the disease during life, and who died from some other cause. The lungs and lymphatic glands are the organs that are usually affected. For purposes of classification we may divide tuberculosis as found at autopsy into three classes: (1) Fatal tuberculosis, in which the disease has been directly responsible for death. (2) *Latent active tuberculosis*, in which advancing, generally caseous, lesions are found, but which do not appear to be the immediate cause of death. (3) *Latent inactive tuberculosis*, in which the lesions are apparently healed and often calcified.

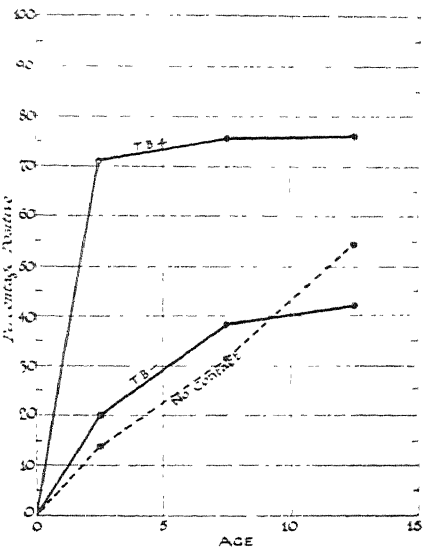


FIG. 277.

Percentage of contact and non-contact children reacting to the intradermal tuberculin test at ages. (Dow and Lloyd 1931).

In 1900 Naegeli published the results of an investigation at Zürich, made to determine the frequency of tuberculous lesions in subjects coming to autopsy. A very careful examination was made of every cadaver, and all doubtful lesions were subjected to microscopic examination. Caseation and calcification were accepted as evidence of tuberculosis; the oblique, distorted indurations at the lung apex were considered tuberculous or not, according to histological examination and the presence of other tuberculous lesions in the body. Table 97 and Fig. 278 show his general results.

The surprising observation was made that, in adults, 93·11 per cent. showed evidence of tuberculosis. In his first series of 136 adults, only 113 or 83·1 per

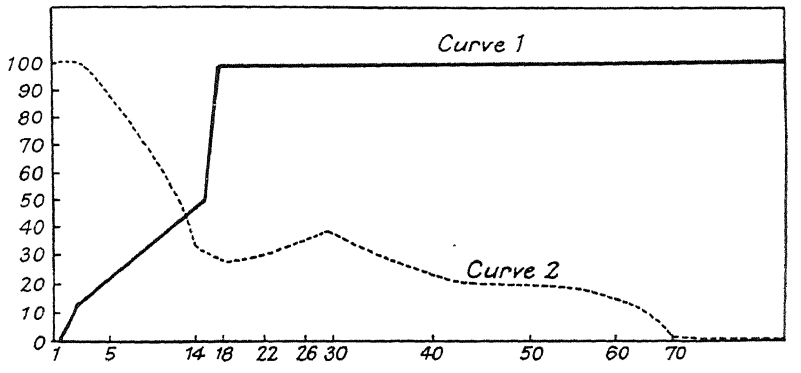


FIG. 278.

Curve 1 = frequency of tuberculous lesions in every 100 autopsies of the same age group.
Curve 2 = frequency of fatal tuberculosis in relation to all cases of tuberculosis.
(After Naegeli.)

TABLE 97

MODIFIED FROM NAEGELI (1900), SHOWING THE FREQUENCY OF TUBERCULOSIS (TB.) OF ALL FORMS IN POST-MORTEM SUBJECTS AT ZÜRICH.

	Autopsies.	Tb. not found.	Tb. found.	Percentage Tb.	Of Tb. Patients.			
					Died of Tb.	Percentage.	Non-lethal Tb.	Percentage.
Children 0–18 years .	88	73	15	17·04	11	73·3	4	26·7
Adults 18 years and over	420	29	391	93·11	110	28·13	281	71·84

cent. were found to be tuberculous; but in his last series of 284 adults, probably owing to his greater experience, as many as 278 or 97·9 per cent. were found to be tuberculous. It will be observed that of the children who showed tuberculosis, 73·3 per cent. had died of the disease (Table 97). In other words, latent or non-lethal tuberculosis is very much commoner in adults than in early life. Naegeli came to the following conclusions: (1) Tuberculosis is very infrequent in infancy, reaches 96 per cent. by the 18th year, and 100 per cent. by the 50th year. (2) In infancy 100 per cent. of cases of tuberculosis are fatal; at 18 years of age 29 per cent. are fatal; 38 per cent. at 30 years; 22 per cent. at 40 years; 10 per cent. at 65 years, and practically none at 70 years. (3) Latent active tuberculosis is uncommon in children; reaches 33 per cent. at puberty; 40 per cent. at 30 years; and falls to 25 per cent. in later years. (4) Latent inactive or healed tuberculosis is infrequent before 18; reaches 25 per cent. in the 3rd decennium; 40 per cent. in the 4th decennium; and 75 per cent. in the 70th year. (5) The disposition towards fatal tuberculosis is therefore highest in infancy; falls to puberty; rises towards 30 years; and later falls again. After 18 years, immunity increases more or less proportionally to age.

A similar, but more extensive series of autopsies, was reported on by Burkhardt in 1906. The material examined came from the Dresden-Friedrichstadt Hospital, and to a less extent from the Kgl. Frauenklinik. It is important to notice that the autopsies were made on the working-class population. Since the mortality from tuberculosis amongst patients of this class was 35 per cent., while amongst the general population of Dresden it was only 13–14 per cent., it is clear that the results he obtained are not representative of the population as a whole. His figures are shown in Table 98.

TABLE 98

MODIFIED FROM BURKHARDT (1906), SHOWING THE FREQUENCY OF TUBERCULOSIS (TB.) OF ALL FORMS IN POST-MORTEM SUBJECTS AT DRESDEN IN 1900–01.

	Autopsies.	Tb. not found.	Tb. found.	Percentage Tb.	Of Tb. Patients.			
					Died of Tb.	Percentage.	Non-lethal Tb.	Percentage.
Children 0–18 years .	190	118	72	38	35	48·6	37	51·4
Adults 18 years and over	1,262	113	1,149	91	466	40·6	683	59·4

These figures agree fairly well with Naegeli's. The chief differences are that the incidence of tuberculosis in children is higher, the mortality from tuberculosis in children is less, and that in adults is higher than in Naegeli's figures. Both agree in showing that in persons of 18 and onwards the incidence of tuberculosis exceeds 90 per cent.

Burkhardt gives a very instructive table recording the incidence of latent tuberculosis in the different age groups (Table 99).

TABLE 99

SHOWING THE FREQUENCY OF LATENT TUBERCULOSIS IN PERSONS CLASSIFIED ACCORDING TO AGE.

Percentages reckoned on every 100 persons of the same age coming to autopsy.

Age Group.	Latent Active Tb.	Latent Inactive Tb.
	Per cent.	Per cent.
1–5	9	4
5–14	25	11
14–18	5	24
18–30	12	26
30–40	14	34
40–50	18	38
50–60	19	44
60–70	21	50
70–80	22	57
80–90	22	61

From this table it is seen that, though the percentage of persons with latent active tuberculosis does not vary greatly in the different age groups, the percentage with latent inactive or healed tuberculosis increases progressively with age.

The general inferences which appear to be justified by these results are as follows:

(1) The great majority of persons living in civilized countries, and particularly in urban communities, have been infected with some form of tuberculosis by the time they attain adult life.

(2) Between 10 and 20 per cent. of persons of all ages living in such communities are suffering from some focus of tuberculous infection which, though latent, is active.

(3) A large proportion of these latent active lesions must heal spontaneously, since evidence of healed tuberculosis increases with age. In parenthesis, it may be pointed out that apparently healed, even calcified, tuberculous lesions may not infrequently contain living tubercle bacilli (Opie and Aronson 1927, Feldman and Baggenstoss 1938, 1939, Saenz and Canetti 1938).

It must be understood that Naegeli's and Burkhardt's figures refer to all forms of tuberculosis. For the frequency of pulmonary tuberculosis, see p. 1314 and Tables 102 and 103 (p. 1314). It would be wrong to assume that the figures of these two workers represent the frequency of tuberculous lesions at the present time. The forty or more years that have elapsed since their observations were made have witnessed a considerable reduction in both the morbidity and the mortality from tuberculosis, so that it is by no means justifiable to say now, as it was then, that practically every adult is infected with the tubercle bacillus. Landé and Wolff (1941), for example, working among a semi-rural population in Maryland,

found that only 48·4 per cent. out of 128 persons over 20 years of age coming to autopsy showed evidence of tuberculous infection.

In Scotland, Blacklock (1932, 1935, 1936) has contributed valuable information on the frequency of tuberculous infection in children. He carried out post-mortem examinations at the Royal Hospital for Sick Children, Glasgow, on 1,800 children under 13 years of age dying from various causes during the years 1924–34. He found (1) that practically all children under 2 years of age showing tuberculous lesions had actually died of tuberculosis, whereas in children 4–13 years of age only about 75 per cent. had died of this disease, and (2) that the percentage of positive tuberculin reactors in the first two years of life was nearly the same as the percentage incidence of tuberculous lesions found at autopsy, indicating again that at this age practically all children who became infected died of the disease. These figures refer to a low social class in Scotland in which tuberculosis is undoubtedly a very severe disease, and it would be unjustifiable to conclude that they are valid for all classes and for all countries. Indeed there is considerable evidence to the contrary (Rosenberg and Kereszturi 1937, Price 1938). With the progressive decline in tuberculosis mortality that is occurring in Europe and America, primary lesions are being increasingly observed in adults. Many of them appear to run a more benign course than those in infancy (Terplan 1934, Wallgren 1938).

In considering the *fatality of tuberculous infection in early life*, it must be remembered that the type of case seen by Naegeli, by Burkhardt and by Blacklock, was not representative of the whole infantile population, but merely of that portion of the infantile population which showed symptoms of disease of sufficient severity to demand admission to hospital. If an equal number of apparently healthy infants could have been examined, it is quite conceivable that many would have shown latent tuberculous lesions. All our figures hitherto have been selective. The diagnosis of tuberculosis in infancy is extremely difficult, and only advanced and fatal cases are recorded. The result is that we have come to believe that infection in infancy is usually fatal. That this conclusion is wrong is shown very clearly by the observations made at Lübeck on the infants who were vaccinated by the mouth during the first few days of life with the B.C.G. strain (see p. 1337). The vaccine had by mischance become contaminated with virulent human tubercle bacilli and a large number of the infants became infected (Report 1935*b*). Out of 251 vaccinated infants, 72 died of tuberculosis, 5 died of other causes, while 174 were alive two to three years later. It is probable that all the surviving children had been infected to a greater or less extent, and radiographical examination actually showed the presence of calcified mesenteric glands in 127 of them. It is interesting to note that practically all cases that developed a primary lung lesion died, while the majority, which had primary alimentary lesions, survived.

We may conclude tentatively that tuberculous infection in infancy is often liable to cause death, particularly if it occurs by the respiratory tract. Infection is, however, by no means uniformly fatal. Provided the environmental conditions are good, and exposure to infection is not too severe or repeated, it seems probable that latent lesions may result of a type similar to those met with in later years.

Latent Pulmonary Tuberculosis as revealed by X-ray Examination.

In recent years X-ray examination of the chest has come to play an important part in the diagnosis of pulmonary tuberculosis, since it is possible by this means to detect lesions before clinical manifestations have developed. In several countries

surveys are now being made to determine the frequency of pulmonary lesions in different classes of the population. Table 100, taken from Hetherington, McPhedran, Landis and Opie (1929), affords an example of the type of result obtained.

It refers to the roentgenological examination of school children, both white and

TABLE 100

SHOWING PERCENTAGE OF PULMONARY LESIONS RECOGNIZED BY X-RAY EXAMINATION IN SCHOOL CHILDREN IN PHILADELPHIA, U.S.A., MOST OF WHOM REACTED TO 1 10,000 TUBERCULIN. (Hetherington *et al.* 1929, and Opie 1930.)

Age Group.	No. of Children Examined	Tb Foci in Lungs and Lymphatic Glands—mainly Calcified.	Childhood Type of Tuberculosis.		Adult Type of Tuberculosis.		Total Lesions.
			Latent Infiltration.	Clinically Manifest.	Latent Apical.	Clinically Manifest.	
5–9 . . .	124	5·9	1·2	0·6	0·0	0·0	7·7
10–14 . . .	452	13·9	2·1	0·5	0·3	0·1	17·1
15–19 . . .	1,060	20·1	1·9	0·2	1·0	0·5	23·7

coloured, in Philadelphia, Pa. The figures are selective, and refer almost exclusively to those children who reacted to an intradermal injection of 0·1 ml. of 1 10,000 tuberculin. It will be noted that in the older children over 20 per cent. showed pulmonary or associated glandular lesions. Of these, 0·7 per cent. actually had clinical symptoms of disease. The distinction between the childhood and the adult type of pulmonary tuberculosis may be neglected for the moment; it will be referred to later (p. 1314).

What proportion of latent lesions progress to a stage at which they give rise to clinical tuberculosis is at present unknown. It is quite clear from the post-mortem evidence that has already been given that many of them must remain latent or gradually retrogress. Special *ad hoc* surveys to follow the history of children with such lesions are at present being made, and it is hoped that in a few years' time we shall be in a better position to answer this question.

Numerous other figures might be quoted to show the high frequency of latent active and overt tuberculosis in medical students and nurses, both of whom are often exposed to an unusually high risk of infection (see Heimbeck 1927, 1932, 1933, Ross 1930, Hetherington *et al.* 1931, 1935, Herman *et al.* 1932, Shipman and Davis 1933, Geer 1934, Kramer 1934, Stiehm 1935, Jacobs 1938, Soper and Amberson 1939, Hart, Hilton and Morland 1940, Hedvall 1940, Hetherington and Israel 1940, Brahdy 1941, Israel, Hetherington and Ord 1941, Alt, Barth and Day 1941, Hastings and Behn 1941, Lyght 1942, Ridehalgh 1942, Daniels 1944). Reference may also be made to similar surveys conducted by X-ray examination, sometimes in association with the tuberculin test, in school children and other classes of the population (see Chadwick 1927, Opie *et al.* 1929, Hewitt and Cutts 1932, Martin *et al.* 1934, Fellows 1934, Rouvillois 1934, Harrington *et al.* 1935, Weber *et al.* 1935, Hetherington, Israel and Kreitz 1938, Pope, Sartwell and Zacks 1939, James 1939, Douglas 1939, Hutchinson and Pope 1940, Galbraith 1941, Trail 1942, Banszky 1942, Clive 1943, Brooks 1944, Marshall 1945).

Frequency of Tuberculosis in relation to Degree of Exposure to Infection.

It has already been pointed out that in children exposed to contact with patients suffering from open tuberculosis the proportion reacting to tuberculin reaches a high figure in the early years of life (see Fig. 277, p. 1303). Observations by numerous workers on families containing one or more members with a positive sputum have now shown that the children in these families have a much higher morbidity and mortality rate from tuberculosis than children in families with sputum-negative patients, and still more so than children in families completely free from tuberculosis. In this country Cox (1929) analysed the histories of 1,486 children under 5 years of age in Lancashire exposed to contact in the home with open tuberculosis. In these children the death rate from non-pulmonary tuberculosis was 9 times greater in the 0–1 year age group, 14 times greater in the 1–2 age group, and 19 times greater in the 2–5 age group than that of a control child population in the County of Lancaster. The excess deaths, it may be noted, were mainly due to tuberculous meningitis. In households containing one or more persons suffering from tuberculosis but having a negative sputum, the death rate of the exposed children was rather greater than that in control children, but the differences were not statistically significant. Cox's figures have received general confirmation from the observations of Turner in Worcestershire (1931).

In the United States even more convincing evidence is available from the extensive observations of Opie (1928–29, 1935) and his colleagues (see Opie and McPhedran 1935, McPhedran and Opie 1935), and of such workers as Evarts, Potter, and Dunn (1934) and Stewart, Gass, Puffer, and Williams (1943). The following figures furnished by Opie, McPhedran, and Putnam (1935), which refer to the development of pulmonary tuberculosis, are of interest in showing not only the much greater frequency of the disease in subjects exposed to open cases of tuberculosis, but also the proportion developing pulmonary tuberculosis in relation to the age of exposure.

(1) Of persons first exposed between 0 and 9 years to contact with sputum-positive patients, 9·92 per cent. of those living 12–14 years after the beginning of exposure had acquired pulmonary tuberculosis, whereas among those exposed to contact with sputum-negative patients, only 1·97 per cent. subsequently developed the disease.

(2) Of persons first exposed between 10 and 14 years to contact with sputum-positive patients, 20 per cent. of those living 10–14 years after the beginning of exposure had acquired pulmonary tuberculosis, whereas none of those exposed to contact with sputum-negative patients had acquired the disease.

(3) Of persons first exposed after 15 years of age to contact with sputum-positive patients, 9·68 per cent. had acquired pulmonary tuberculosis, while the corresponding figure for persons exposed to sputum-negative patients was 6·86 per cent.

On the basis of these observations we may conclude with Opie and his colleagues that the spread of tuberculosis in the community is, in great part, the result of slowly progressive household epidemics, which often lead to the transmission of the disease by contagion from one generation to another.

TUBERCULOSIS OF HUMAN ORIGIN. METHODS OF INFECTION

Tubercle bacilli may gain entrance to the body by various routes.

The possibility of *congenital* infection seems to be very small. Sitzenfrey (see Ghon *et al.* 1925) examined 26 placentas from tuberculous mothers, and found tuberculous lesions in 7 of them ; on the basis of these results Moll (see Ghon *et al.* 1925) argues that there are two types of infantile tuberculosis : (1) an early form due

to hæmatogenous infection of the fœtus from the placenta; and (2) a later form, of respiratory origin, occurring in extra-uterine life. The former type he would regard as definitely congenital. But Ghon, Kudlich and Winternitz (1925), who investigated this subject, found no evidence to support the occurrence of congenital infection; in 87 infants investigated at autopsy they found a primary focus of disease indicative of post-natal infection; in none of them was a picture found corresponding to Moll's early form.

Tubercle bacilli may gain access to the body by *direct inoculation*, as by an infected needle or knife, or through an abraded pustule (von Eiselberg 1887). Cases of direct inoculation of tuberculosis are uncommon, but are seen occasionally in butchers, post-mortem attendants, and laundresses.

Infection may occur by passage of the bacilli through the various mucous surfaces of the body, but except for that part of the mouth and pharynx which is more or less common to the respiratory and alimentary tracts, this route of infection is probably of minor importance.

Pulmonary Tuberculosis.—We now come to the much-discussed question of the mode of infection in the most common of all tuberculous diseases—pulmonary tuberculosis. There are two main hypotheses: the first, advocated by von Behring and by Calmette and his colleagues, maintains that infection occurs primarily through the mucosa of the alimentary tract; the second, advocated by Cornet, Flügge, and in this country by A. S. Griffith and Cobbett particularly, maintains that infection occurs primarily by the respiratory tract. We must consider briefly the evidence in favour of each of these opposing views.

Von Behring in 1904 advanced the hypothesis that the pulmonary tuberculosis of adolescent and adult life is the end-result of an infantile infection contracted by the absorption through the mucosa of the alimentary tract of bacilli ingested with the food. In childhood the infection is manifested by caseous glands, particularly cervical and mesenteric; in adult life by caseous apical tuberculosis of a chronic type. This hypothesis was supported to some extent by the work of Calmette and Guérin (1905).

Pregnant goats were injected into each udder with 0·02 gm. of a fresh bovine culture. Severe lesions resulted, confined to the udder, and proving fatal in 40–60 days. From one such animal two kids were raised; one of these was killed in a sickly condition on the 45th day, and the other died on the 51st day after birth. This second animal, besides showing enlarged mesenteric glands at necropsy, also had miliary tubercles in the lungs; the peribronchial glands were normal. In a second experiment, performed on two kids 5 days old, they delivered 0·05 gm. of a fresh bovine culture directly into the stomach; two or four doses were given. One of the animals died about 10 weeks later; post mortem, the mesenteric glands were greatly enlarged; the lungs were riddled with tubercles; and the peribronchial and posterior mediastinal glands were very large and were filled with caseous tubercles. In a third experiment, the same procedure was adopted on adult goats. When killed 7 to 9 weeks later, these animals showed little or no swelling of the mesenteric glands; the peribronchial and posterior mediastinal glands were not affected; but the lungs were tuberculous. On the basis of these results they conclude that, in the great majority of cases, pulmonary tuberculosis is contracted, not by inhalation, but by ingestion of dust or products containing tubercle bacilli; the bacilli pass through the mucosa of the alimentary tract and are carried to the lung. They dissent, however, from von Behring's suggestion that adult pulmonary tuberculosis is due to the slow evolution of an intestinal infection contracted in infancy: on the contrary, they think that adults develop pulmonary tuberculosis more easily than children after intestinal infection;

children are better protected by their lymph-glandular system. Dust, they consider, is infective, not because it is inhaled, but because it is swallowed.

Further experiments by Calmette and Guérin (1906*a*, *b*) showed that the lungs might become diseased after intra-œsophageal infection of heifers and cows with bovine bacilli. Vallée (1905) found that the bronchial glands of young calves might be infected by feeding the animals on tuberculous milk, but he failed by this method to produce lesions in the lungs. One further piece of evidence adduced by Calmette in favour of the intestinal origin of infection in tuberculosis is the analogy presented by glanders. He states that it is impossible to produce the usual pulmonary type of glanders in horses by intratracheal injection of the bacilli; but if the glanders bacilli are given to the horse in its drinking water, pulmonary glanders consistently results. He concludes (1920) that in all susceptible animals, including man, tuberculosis in its manifold varieties of localization—glandular, pulmonary, alimentary, etc.—results in the great majority of cases from an infection, primarily lymphatic, then hæmatogenous, having its origin in the absorption of tubercle bacilli by the digestive tract—principally by the buccal, pharyngeal, and intestinal mucosæ.

It will be realized that the evidence adduced by Calmette is derived entirely from experiments on animals. There is no doubt that pulmonary lesions may follow the alimentary infection of goats and heifers; but the observations of White and Minett (1941) in the calf suggest strongly that the bacilli reach the lungs not through the intestine but by aspiration down the trachea during the process of feeding or intra-œsophageal inoculation. When the bacilli were introduced directly into the duodenum, so as to exclude regurgitation and aspiration, the lungs never became infected.

The evidence in favour of the respiratory hypothesis, on the other hand, is derived largely from observations made on human patients; it depends partly on the anatomical distribution of the lesions, and partly on the types of infecting bacilli.

The workers on the Royal Commission, and subsequently A. S. Griffith, Eastwood, and F. Griffith, examined in all 260 children under 12 years of age who had died of tuberculosis. From 32 of these children, bovine tubercle bacilli were isolated. In all the cases infected with bovine bacilli in which there were lesions, the anatomical evidence pointed definitely to the alimentary tract as the portal of entry of the bacilli (Griffith, A. S., 1925*b*). In these cases the mesenteric glands were the most severely affected regional lymphatic glands; the bronchial glands either were not macroscopically tuberculous, or showed disease of slighter degree and in an earlier stage. The anatomical picture of the disease in children from whom human tubercle bacilli were obtained was less uniform than in the bovine cases. They could be divided into three groups: (1) In the first group the respiratory tract was evidently the portal of entry; the bronchial glands were the most severely affected regional lymphatic glands; the mesenteric glands either not being affected, or showing more recent disease obviously secondary to the thoracic disease. (2) In the second group, both the bronchial and the mesenteric glands were severely affected, and there was nothing to indicate that one set of glands had been infected previously to the other. Probably infection had occurred simultaneously by both the respiratory and the alimentary tracts. (3) The third group was a small one, and contained those cases in which the alimentary tract was the sole portal of entry of the human bacillus; the distribution of disease was the same as in the bovine cases.

From these results the following conclusions were drawn: (1) The path of infection of the *bovine* tubercle bacillus is almost exclusively the alimentary tract. (2) The chief portal of entry of the *human* tubercle bacillus is the respiratory tract. (3) Primary abdominal tuberculosis due to the human tubercle bacillus is rare.

We know that pulmonary tuberculosis is, with few exceptions, due to the human tubercle bacillus ; and we have evidence, founded on post-mortem results, to show that the human tubercle bacillus generally enters the body by the respiratory tract. We may therefore conclude that in most cases of pulmonary tuberculosis infection has occurred by the respiratory and not by the alimentary tract.

Further evidence of the inhalation hypothesis is obtained by considering the types of tubercle bacilli that are found in different situations of the body (Table 101).

It will be seen that 98 per cent. of infections of the lungs and 95 per cent. of infections of the bronchial glands are due to the human type of bacillus. On the other hand, as many as 50 per cent. of abdominal and 46 per cent. of cervical gland infections are due to the bovine bacillus. If, as von Behring and Calmette suppose, pulmonary tuberculosis were due to bacilli that had gained access to the body through the alimentary tract, it would be reasonable to expect that about 50 per cent. of infections of the lungs and bronchial glands would be due to the bovine bacillus. This is clearly not the case. The figures in the table afford no evidence that the bacilli pass downwards from the cervical, or upwards from the mesenteric, glands to invade the bronchial glands and lungs ; on the contrary, the fact that bovine bacilli are uncommon in these organs suggests that infection must occur by some other route, namely the respiratory tract.

TABLE 101

SHOWING THE PROPORTION OF BOVINE AND HUMAN INFECTIONS IN DIFFERENT VARIETIES OF TUBERCULOSIS. (Modified from Cobbett 1922.)

Type of Tuberculosis.	Type of Tubercle Bacillus. England. All Ages.	
	Human.	Bovine.
	Per cent.	Per cent.
Pulmonary	97·9	2·1
Bronchial glands	94·5	5·5
Abdominal	50	50
Cervical glands	54	46

If the lungs are infected by the respiratory tract it is not difficult to understand why bacilli of the human type should almost invariably be found in pulmonary tuberculosis. The most common source of infection with the bovine type is milk, and this is invariably ingested by the mouth ; human tubercle bacilli, on the other hand, are often present in the air in the form of cough-spray or in dust from dried sputum, and are in a suitable state to be inhaled. The almost invariable presence of human bacilli in tuberculous lungs strongly suggests that infection occurs by inhalation.

Another argument in favour of the respiratory mode of infection in pulmonary tuberculosis is based on a comparison of the frequency of mesenteric tuberculosis in this country and America. Examining 67 young British adults, Opie (1925) found healed tuberculous lesions of the mesentery in 27 per cent.—a finding that he ascribes to the high incidence of bovine tuberculosis in this country. In America, where bovine tuberculosis is much less frequent, lesions of the mesentery are uncommon in human beings. Yet both in this country and in America, pulmonary tuberculosis is very frequent. If the lungs were infected from the alimentary tract, pulmonary tuberculosis should be much commoner in this country than in America. But this is not so.

Finally we may refer to the very careful studies of Ghon (1916) in Vienna, and of Opie (1917*a*, *b*, 1924*a*, *b*, 1925) in America, on the anatomical distribution of the lesions

in the pulmonary tuberculosis of childhood. Dissection of the lungs after death, aided by preliminary examination with the X-rays, showed that the primary lesion occurred in the substance of the lungs, and that the bronchial glands were infected only secondarily. This clearly points to the respiratory tract as the portal of infection. If the bacilli reached the lungs from the alimentary tract *via* the lymphatics, the bronchial glands should show the primary, and the lungs the secondary lesions.

From these various sources of evidence we conclude that, with the exception of those cases of miliary tuberculosis in which the lungs have been infected from the blood stream, the vast majority of cases of pulmonary tuberculosis are due to infection by the respiratory tract. In the light of recent work a further exception should perhaps be made for those cases of tuberculosis of bovine origin in which the pulmonary disease is apparently secondary to lesions in the glands. Further work is required on the exact mechanism of infection in these cases, but there is no reason to modify substantially the general conclusions that have just been reached.

Dust and Droplet Infection.

Accepting the inhalation hypothesis, we are faced with the problem of how the bacilli gain entrance to the respiratory tract. The evidence brought to bear on this problem is so extensive that we can give nothing more than a brief outline of the conclusions drawn from it. According to Cornet (1889), the chief source of infection is the *dust arising from dried sputum*. Examining 140 specimens of dust taken from various situations—chiefly hospitals, public buildings, and the private rooms of tuberculous patients—he found tubercle bacilli in 40 of them. He believed that tubercle bacilli may remain alive and virulent in dust for 3 or 4 months. He maintained that, apart from his sputum, a patient suffering from pulmonary tuberculosis is harmless. The main objections to this view are: (1) For the tubercle bacilli to be set free by the desiccation of sputum the air must be absolutely dry; if it is at all moist, complete desiccation does not occur. In Europe, at any rate, the atmospheric conditions during most of the year are unfavourable for complete desiccation. (2) During the process of drying there is evidence that the tubercle bacilli, especially if exposed to sunlight, are liable to die (Heymann 1901, Mayer, E., 1921, Caldwell 1925, Eidinow 1927).

According to Flügge (1899) and members of his school (Moeller 1899, Heymann 1901, Findel 1907, Ziesché 1907, Hollmann 1924), the tubercle bacilli gain entrance to the respiratory tract in the form of *minute droplets* expelled from the mouth and nose of patients suffering from open pulmonary tuberculosis. Buchner showed that bacteria suspended in a very fine spray could be conveyed through several metres of rubber tubing, even when only a light current of air was used. Laschtschenko found that sprayed tuberculous sputum carried tubercle bacilli in suspension over a considerable distance when propelled by an air-current of only 3 mm. per second. By taking suspensions of *Chr. prodigiosum* into his mouth, he showed that organisms were expelled to a slight extent during speaking, to a greater extent in coughing, and to a still greater extent in sneezing. Numerous workers have found that tuberculous patients when coughing expel droplets containing tubercle bacilli; these droplets are projected for varying distances, but not further as a rule than 1½ metres. Guinea-pigs placed about half a metre from the mouth of tuberculous patients, and exposed freely to their cough-spray, not uncommonly contract tuberculosis.

To this view Lange and Keschischian (1925) raise objections on the ground that the droplets expelled during the act of coughing are so large that they rapidly fall to the ground.

Using a Buchner spray they found that droplets of an aqueous solution of eosin of 200 μ or over in diameter remained in suspension for a few seconds; droplets of 20 μ and under for a few minutes, and droplets of only a few μ for 1 to 3 hours. But since the droplets expelled in coughing are said to be fairly large, they conclude that primary lung infection by droplets must be uncommon. Their measurements of the size of the droplets were made on glass slides; but as Strauss (1926) points out, the diameter of a droplet that has settled on a slide is not the same as its diameter when suspended in the air; in fact it is about three times as great. He concludes, from a series of measurements he made, that the diameter of droplets of sputum suspended in the air is about 50–250 μ, the majority being 70–85 μ. Droplets of this size can probably remain in suspension for a minute or two, and are consequently liable to be inhaled.

W. F. Wells (1934) notes that the falling velocity of a small droplet is proportional to the square of its diameter. He calculates that the time taken for droplets of various sizes to fall 2 metres—rather over the height of a tall man—is as follows: droplets with a diameter of 0·1 mm. 6 seconds, of 0·01 mm. 10 minutes, of 0·001 mm. 16·6 hours. But as these droplets fall they evaporate, and the rate of evaporation is inversely proportional to the square of the diameter. Water droplets with a diameter of 1 mm., falling in unsaturated air at a temperature of 18° C., will evaporate in 165 secs., those with a diameter of 0·1 mm. in 1·7 secs., those with a diameter of 0·05 mm. in 0·4 secs. Droplets expelled from the human mouth in coughing, sneezing or loud talking will clearly not evaporate so rapidly as droplets of water, since they contain in solution substances that tend to retain water; but the same general relations will hold. The larger droplets, those of 0·1 mm. or over in diameter, will tend to behave as above. They will settle rapidly, and while settling, and for some little time after, they will retain their droplet form. Smaller droplets will behave quite differently. They will remain suspended in the air for much longer periods; but, since they soon lose their water by evaporation, they will quickly cease to be droplets and will become what the author refers to as "droplet nuclei," *i.e.* the bacteria or other particles contained in the droplet together with any substances in solution, the whole in a desiccated or semi-desiccated state. The physical properties of these "droplet nuclei" are not precisely calculable, but all analogies suggest that they might remain suspended in air for a very considerable time. They would, moreover, be carried long distances by air currents.

This wide difference in behaviour between large and small droplets is, as the author emphasizes, of obvious significance in relation to the spread of infection. The term "droplet infection," as commonly employed in medical literature, should be regarded as applying to the spread of infection by droplets larger than 0·1 mm. in diameter. It is spread of this sort that will usually be measured in experiments where Petri dishes are exposed at various distances from a coughing or speaking person. Spread by droplets smaller than 0·1 mm. in diameter and by their nuclei is best described as "air-borne" infection. It will obey quite different laws and may, under certain conditions, be of great epidemiological importance. (See also Wells and Stone 1934, Wells and Wells 1936, and Chapter 91.)

From animal experiments there is no doubt that tubercle bacilli, whether in the form of dry dust, of moist droplets, or of droplet nuclei, can penetrate the respiratory passages and reach the lung (Lange and Nowoselsky 1925, Wells and Lurie 1941, Glover 1944). It is probable therefore that Cornet and Flügge are both right. The only question is which mode of infection is the more common—dust or droplet infection. Considering that in this country the atmospheric conditions are seldom suitable for the formation of absolutely dry sputum, whereas the opportunities for droplet infection must be extremely common in such situations as trains, 'buses, trams, theatres, meeting-places, and crowded rooms of all sorts, droplet infection, including air-borne infection by droplet nuclei, is probably of far greater importance

than infection by sputum. The discussion may seem to be of purely academic interest; but from the standpoint of preventive medicine it is of considerable importance. Injunctions against spitting are numerous; but if Flügge's view is correct, it is clearly of far greater value to educate the public never to cough or sneeze without putting a handkerchief in front of the mouth. In this country a start has been made along these lines. It is to be hoped that the result will lead to a diminution in the risk of contracting not only pulmonary tuberculosis but all diseases that are spread by the respiratory tract. Some idea of the degree of infection of the air may be gathered from the observations of Pressman (1937) in the United States who, by the simple expedient of exposing four dishes containing saline in the corners of a room for one week, was able to demonstrate the presence of tubercle bacilli in 87 per cent. of 55 samples taken from four tuberculosis hospitals and sanatoria.

Pathogenesis of Pulmonary Tuberculosis. Childhood and Adult Types.

Evidence has already been brought to show that pulmonary tuberculosis of adults is usually due to respiratory infection. We must now consider the different types of pulmonary disease that occur in childhood and adult life, and decide whether adult pulmonary tuberculosis is a continuation or recrudescence of the childhood form, or is due to a re-infection in later years.

As previously stated, Ghon (1916) came to the conclusion that the primary lesion in tuberculosis of infants and children occurs in the substance of the lungs, and that secondary to this infection the adjacent lymphatic glands become involved. Opie (1917*a*, *b*, 1924*a*, *b*, 1925), besides confirming this conclusion, carried out a large series of examinations on the lungs of persons of all ages who had died of non-tuberculous diseases. The pulmonary tuberculosis of children up to 10 years of age was, he found, essentially of a *focal* type; the lesions were situated in any part of the lungs, and were accompanied by caseation or calcification of the regional lymphatic glands. In older children, and in adults, two types of tuberculous lesions were found: focal lesions, similar to those in infants and young children; and apical lesions, situated at the apex of the lungs, and not accompanied by caseation of the regional glands. Focal lesions developed in early childhood, and increased in frequency throughout life, as can be seen by reference to Table 102.

TABLE 102

FREQUENCY OF HEALED TUBERCULOUS LESIONS (FOCAL OR APICAL) IN THE LUNGS OF PERSONS IN ST. LOUIS, DYING OF DISEASES OTHER THAN TUBERCULOSIS.

Age.	Frequency.
2–5 years	42·8 per cent.
5–10 „	45·5 „
10–18 „	55·5 „
18–30 „	83·3 „
30–50 „	91·3 „
50–70 „	93·3 „

Apical tuberculous lesions were not encountered in young children, but increased with advancing age (Table 103).

TABLE 103

FREQUENCY OF LATENT APICAL TUBERCULOSIS IN 50 AUTOPSIES ON PERSONS IN ST. LOUIS, DYING OF DISEASES OTHER THAN TUBERCULOSIS.

Age.	Frequency.
18–30 years	16·7 per cent.
30–50 „	13 „
50–70 „	26·7 „
70 and over	50 „

The question naturally arises: Have the apical lesions of adult life developed from the focal lesions of childhood? Opie brings evidence to disprove this: (1) apical tuberculosis is more common in later life when the focal lesions of childhood have in most instances completely healed; (2) in adults the focal lesions of childhood are almost invariably calcified and healed, and no longer contain living tubercle bacilli (Opie and Aronson 1927), whereas the apical lesion is relatively fresh and caseous; (3) in at least half of the cases examined, the focal lesion was in one lung and the apical lesion in the opposite lung. Moreover, the characters of active pulmonary tuberculosis in childhood and in adult life are different.

Characters of Active Pulmonary Tuberculosis of Childhood.	*Characters of Active Pulmonary Tuberculosis of Adults.*
1. No particular localization.	1. Apical localization.
2. Hilum glands involved.	2. Hilum glands not involved.
3. Little or no tendency to cavity formation or deposition of fibrous tissue.	3. Tendency to cavity formation and deposition of fibrous tissue within the lung substance.
4. Rapid course.	4. Chronic course.
5. Similar to infection of normal guinea-pig.	5. Similar to infection of tuberculous guinea-pig.

We may conclude therefore that, though in most cases of apical tuberculosis a focal lesion is present, the apical lesion is not an extension of the focal disease. In other words, the phthisis of adults is not the result of tuberculosis acquired in childhood.

Additional evidence in support of this view has been advanced by Opie (1928–29, 1935) and by Opie, McPhedran, and Putnam (1935). Following up children exposed to contact with open tuberculosis at some time between birth and 9 years of age, these workers found that the maximum proportion of infiltrating lesions of the childhood type detectable by X-ray examination was reached at about 3 years of age, after which it fell gradually to zero by 11 years of age. On the other hand, latent apical lesions of the adult type were not seen before 4 or 5 years of age, but after this they rose progressively as the age of the child increased. In other words, the two types of lesions behaved as if they were entirely unrelated. Further observations showed that children first exposed to infection after 15 years of age usually developed the typical adult apical type of pulmonary disease.

All these findings suggest that the pulmonary tuberculosis of adults is not usually a continuation of childhood disease, but is due to a re-infection some time during late childhood or early adolescent life. It must be pointed out, however, that the lesions so produced often remain latent, and clinically manifest tuberculosis may not develop for months or years after re-infection.

Endogenous and Exogenous Infection.

The long period during which it was taught that practically everyone reaching adult life had already been infected with the tubercle bacillus was characterized also by the belief that pulmonary tuberculosis of the adult type was the result either of activation of an old pulmonary lesion or of endogenous superinfection. It is now realized that, except in crowded urban communities, a proportion of persons may reach adult life without becoming infected, and more attention is therefore being devoted to the part played by exogenous infection in the pathogenesis of pulmonary tuberculosis. It is known that adults who become infected for the first time may develop a form of pulmonary tuberculosis indistinguishable from that occurring in those with a recognizable healed primary lesion, suggesting that, though the peculiar nature and course of adult pulmonary tuberculosis may be determined to some extent by experience of a previous infection, the natural resistance of the primarily infected healthy adult may alone suffice to bring about the same result.

The main problem concerns the genesis of pulmonary tuberculosis in those who have already been infected during childhood. Is the disease due to endogenous or exogenous superinfection? As invariably happens when the data are insufficient, there is a sharp cleavage of opinion on this point. Since Opie's work, however, the protagonists of the exogenous view have been gaining ground. We have no space here to discuss the evidence in detail; reference to one or two representative papers must suffice.

There is a wealth of evidence to show that pulmonary tuberculosis is much commoner among family contacts than among non-contacts (see p. 1302). Moreover Lloyd and Macpherson (1936) found that about 4 out of every 5 young adults suffering from pulmonary tuberculosis who had been in contact with a known case of infection developed the disease within five years of the last known exposure—a time relationship that is difficult to understand if the disease was of endogenous origin. Observations by Plunkett, Weber, Siegal and Donk (1940) in the Newark State School for Mental Defectives showed that during a period of three years 13 new cases of pulmonary tuberculosis developed among tuberculin-positive patients. Every one of these had been in close contact with an infectious case. Among those who had not been exposed to known infection, not a single case was reported. Israel and DeLien (1942), who studied the fate of 932 persons of 14 years or over who had had a positive tuberculin reaction during childhood, found that in white persons the mean annual morbidity rate among those exposed to household infection after 14 years of age was 2 per cent., as against only 0·1 per cent. in those with no household exposure. The morbidity rate, however, in those with radiographical evidence of a primary tuberculous lesion was, in the absence of household exposure, not significantly higher than in those without such a lesion. The authors conclude therefore that pulmonary tuberculosis in adolescence and early adult life is more often the result of household exposure after childhood than before, indicating that in early adult life exogenous infection is more important than the endogenous reactivation of lesions acquired in early childhood.

Whether exogenous superinfection gives rise to pulmonary disease directly, or whether, as Wingfield (1942) suggests, it brings about an endogenous exacerbation by causing a focal tuberculin reaction, will be impossible to decide till we know a great deal more about the part played by allergy in the mechanism of immunity to tuberculosis (see pp. 1330–1336).

If it is true that exogenous infection in adult life may result in pulmonary tuberculosis, then far more care should be exercised than has hitherto been general

in protecting not only children but adults, even tuberculous adults, against the risk of exposure to infection. There is still a widespread tendency to neglect or minimize the risk of adult infection. In the absence of strong evidence to the contrary, we consider this attitude to be dangerous and reprehensible. Experience of the control of infectious disease, whether of bacterial or virus origin, has established the principle that avoidance of infection constitutes the first line of defence. Vaccination by natural or artificial means plays, as a rule, a much less important rôle. We see no reason why tuberculosis should be regarded as different in this respect from other diseases.

TUBERCULOSIS OF BOVINE ORIGIN. FREQUENCY AND MODE OF INFECTION

Frequency.—The frequency of this type of tuberculosis varies greatly in different countries and in different parts of the same country, depending in general on the incidence of tuberculosis in cattle and the quantity of raw milk consumed. In Scotland the percentage of bovine infection in all forms of tuberculosis is higher than in England. Taking Great Britain as a whole, there were in 1931 about 2,600 deaths and 6,250 notifications (Report 1934). Of the deaths, about three-quarters occurred in children under 15 years of age. These figures are calculated on the proportion of bovine infections in different forms of tuberculosis, and are subject to the error of random sampling. Making allowance, however, for such an error, and for the fact that the notifications probably underestimate the true number of cases, it is clear that the bovine tubercle bacillus is responsible in Great Britain for a large number of deaths and for an immense amount of suffering, invalidity, and often permanent deformity.

In England and Wales about 6·0 per cent. of all deaths from tuberculosis are due to infection of bovine origin. The frequency in other countries is less certain. In the United States, Park and Krumwiede (1910), who examined 436 strains of tubercle bacilli, of which 291 were derived from patients with pulmonary tuberculosis, found that 7·57 per cent. were of the bovine type. This percentage, however, is not applicable to the present frequency of tuberculosis of bovine origin. Since 1917 an intensive campaign has been carried out under the Federal Government to eradicate tuberculosis in cattle, and the extent to which these animals are infected is now on an average probably only about one per cent. of that in Great Britain. Clinical observers are unanimous in agreeing on the comparative infrequency in the United States of cervical and mesenteric glandular tuberculosis in young children, both of which manifestations in this country are generally due to the bovine bacillus (see Reichle 1936). In Germany, Austria, Denmark, Sweden, Poland, and the Netherlands the disease appears to be fairly frequent, though perhaps less so than in Great Britain (see Klimmer 1931, Lange 1932, 1937, Blacklock 1932, Tobiesen *et al.* 1935*a*, *b*, de Lange and de Bruin 1935, Piasecka-Zeyland 1937, Ruys 1937, van der Hoeden 1937, van der Hoeden and Pannevis 1937, Hedvall 1942.)

Source of Infection.—Cattle form the great reservoir of infection with the bovine bacillus. In this country about 40 per cent. of all milch cows react to the tuberculin test; about 40 per cent. slaughtered in public abattoirs show naked-eye lesions of tuberculosis; and about 0·5 per cent. are actually excreting tubercle bacilli in their milk (Report 1932*a*, 1934, Savage 1933). The proportion of milk samples containing virulent tubercle bacilli varies with the nature of the sample and the part of the

country from which it is derived. On an average about 7 per cent. of samples of mixed milk from individual farms contain tubercle bacilli, the proportion varying from about 2 per cent. in Somerset to about 21 per cent. in Cheshire (Report 1932*a*, Gloyne 1932, Pullinger 1934, 1935, Parry and Hall 1935). In Scotland the proportion seems to be even higher (Report 1933*a*).

We have no space to furnish the complete evidence incriminating milk as the main cause in man of tuberculosis of bovine origin. The general nature of the evidence rests on (1) the parallelism in different countries between the frequency of tuberculosis in cattle and of tuberculosis of bovine origin in man ; (2) the high proportion of alimentary infections in man due to the bovine type ; (3) the frequency of non-pulmonary tuberculosis of bovine origin in infancy and childhood ; (4) the comparative infrequency of tuberculosis of bovine origin in areas in which the majority of the milk, even though heavily infected, is pasteurized or boiled before use ; (5) the production of tuberculosis in animals fed on infected milk (Griffith 1907, Wilkie *et al.* 1937). The most convincing evidence is afforded by the observations of Price (1932, 1934) in Toronto on the frequency of the bovine type of bacillus in non-pulmonary tuberculosis of childhood. Of 300 tuberculous children examined under 14 years of age, 15 per cent. were found to be infected with the bovine type. Without exception, these children came from parts of Ontario outside Toronto where pasteurization of milk was not carried out, and all had a history of being fed for some time on raw milk. In Toronto, where pasteurization has been compulsory since 1915, not a single case of tuberculosis of bovine origin was found, in spite of the fact that 26 per cent. of pooled samples of raw milk coming into the town contained tubercle bacilli.

The bacilli in the milk may pass through the lymphoid tissue of the mouth and nasopharynx, and reach the cervical glands, where they give rise to disease. The tonsil seems to be not uncommonly infected (Saenz *et al.* 1933). Alternatively they may pass through the intestinal mucosa and invade the mesenteric group of glands. There seems to be little doubt that the organisms may traverse the mucous membrane without giving rise to any lesions in the mucosa itself. Once they have gained access to the lymphatic system, they may be distributed in the body either by direct spread, by the lymph stream, or by the blood stream. Blood-borne infection appears to be mainly responsible for miliary tuberculosis, meningitis, bone and joint disease, and genito-urinary tuberculosis.

Pulmonary tuberculosis of bovine origin is mainly a rural disease. On this point both British and continental workers are in agreement (Lange 1937, Griffith 1937*a*, 1938, 1941, Ruys 1937, van der Hoeden 1937, Griffith and Smith 1940, Hedvall 1942, Cutbill and Lynn 1944). The mode of infection, however, is still open to dispute. Most of the work in this country suggests that the disease follows most commonly on glandular tuberculosis acquired through alimentary infection, but in Germany Lange (1931, 1932) has brought evidence to show that in farm workers infection may result from the inhalation of tubercle bacilli expectorated by tuberculous cattle (see also Hedvall 1942). In cases following on primary glandular infection the incubation period ranges from less than a year to 26 years or more (Griffith 1938). Griffith (1937*a*) regarded the disease as the final phase of an infection acquired in childhood, adolescence, or even adult life through the consumption of tuberculous milk. Occasionally infection is transmitted from one person to another by contact (Griffith 1938). The clinical course of the disease is often rapid and a considerable proportion of cases prove fatal.

Summarizing, we may say that practically all non-pulmonary, and a variable proportion of pulmonary, cases of bovine origin are contracted through the agency of raw milk or cream. *Cheese* and *butter*, it may be noted, probably play an insignificant part in the transmission of infection in Great Britain. Over 80 per cent. of butter is imported and practically all imported butter is made from pasteurized cream. In cheese the tubercle bacillus dies out fairly quickly, and is usually dead before the ripening process is complete. Only in farm butter and in soft cheese, *i.e.*, unripened cheese eaten within a few days of its preparation, are tubercle bacilli likely to be found alive.

It is of interest to recall the changes that our views on this type of tuberculosis have undergone in the present century. At the London Congress on Tuberculosis in 1901, Koch asserted, on the basis of quite inadequate experimentation, that bovine bacilli were virtually non-pathogenic to human beings. His views were attacked by McFadyean (1901) and by Ravenel (1901) on the basis of strong, though circumstantial, evidence. As the result of considerable opposition, Koch modified his views at the Washington Congress in 1908, to the extent of admitting that human beings might be infected with bovine tubercle bacilli, though he maintained that serious disease due to these organisms was very rare, and that preventive measures to protect the human population against tuberculosis of bovine origin were quite unnecessary. The extensive labours of the Royal Commission on Tuberculosis in this country, and of continental and American workers, revealed the fallacy of Koch's teaching, and showed that non-pulmonary tuberculosis in childhood was frequently caused by the bovine bacillus. The more recent work of Griffith, Munro, Lange, and others has now drawn attention to the occurrence of bovine bacilli in pulmonary tuberculosis; and infections of bovine origin as a whole must be regarded as a serious menace to the human population in those countries where tuberculosis of cattle is common. The evidence suggests that the bovine bacillus is quite as virulent for man as the human bacillus, and possibly even more so (see Griffith 1925*b*). The result of Koch's teaching was of more than academic importance. By exonerating cows from all blame in the causation of tuberculosis in man, it put back the clock of veterinary preventive medicine to a degree far greater than is generally appreciated. It further removed the rationale for boiling milk, which had hitherto been accepted on empirical grounds, and delayed for a long time the adoption of pasteurization. Even now we are not free from its trammels, and the public health expert has to fight a continuous battle against the ignorance and prejudice resulting from an authoritative statement made on inadequate evidence 45 years ago. (For general review of tuberculosis of bovine origin, besides those already quoted, see Griffith 1927, 1932*c*, 1937*a*, 1938, 1941, Report 1931*b*, Gervois 1937, Myers 1940, Wilson 1942.)

DIAGNOSIS

Microscopical Examination in Diagnosis.—Tuberculosis is one of the few diseases in which a diagnosis can often be made by microscopical examination alone. The simplest method is to stain a film of sputum, pus, or other pathological product with Ziehl-Neelsen's stain, using 25 per cent. sulphuric acid for decolorization, and 1 per cent. aqueous methylene blue for counterstaining. Numerous other methods of staining, however, have been recommended from time to time, such as Spengler's

(1907), Herman's (1908), Mori's (1911), Bozzelli's (1914), Schulte-Tigges' (1920), and Pottenger's (1942). When very few organisms are present, attempts to increase their numbers or to render their distribution in the film more uniform may be made by (*a*) chemical concentration methods, such as the antiformin method or the methods described by Faisca (1921), Grysez and Bernard (1920), G. Mayer (1926), and S. R. Douglas and Meanwell (1925); (*b*) chemical flocculation methods, such as that of Hanks, Clark and Feldman (1938); (*c*) flotation methods, such as those described by Andrus and MacMahon (1924), Pottenger (1931), Edwards, Lynn and Cutbill (1936), C. R. Smith (1938), and Hanks and Feldman (1940).

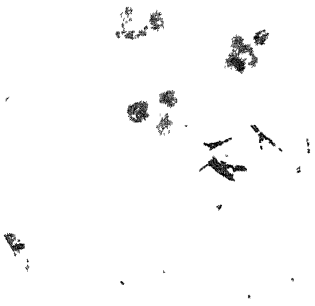


FIG. 279.—*Mycobacterium tuberculosis*, in urine, showing the characteristic clumps (× 1000).

Urine should be centrifuged, and films made from the deposit (Fig. 279). When examining the cerebrospinal fluid, it is best to allow the fluid to stand till a fibrinous clot forms; this should be removed with a platinum loop, spread on a slide, and stained in the usual manner for acid-fast bacilli. Otherwise it should be centrifuged and treated like urine.

In both pulmonary and non-pulmonary tuberculosis the bacilli may often be found in the fæces (Alexander 1910, Costa 1926, Sergent 1926, Sykes 1926, Verdina 1926); for their detection some concentration method, such as the ligroin method, should be employed. Examination of the fæces is chiefly of use in children, who generally swallow their sputum, and in cases of closed pulmonary tuberculosis in which there is no sputum. It has been suggested that the bacilli may be excreted from the blood into the bile, and thus reach the fæces (Alexander 1910, Calmette 1920, Remlinger 1923, Koizumi 1924, Verdina 1926).

Examination of the fasting stomach contents obtained by gastric lavage is used extensively in the diagnosis of pulmonary disease in children, and in adults with little or no sputum (Kereszturi *et al.* 1932, 1933, Collis and Brockington 1933, Poulsen and Andersen 1934, Gourley 1934, Nalbant 1934, Stadnichenko, Cohen and Sweany 1940, Webster 1941, 1943, Davies and Doherty 1942, Robinson and Dunn 1943). The method was described originally by Meunier in 1898, but was not adopted as a routine till it was rediscovered by Armand-Delille (1927) nearly 30 years later. To avoid the mild unpleasantness of the passage of a stomach, or preferably nasal, tube, simpler methods of obtaining sputum have been described by Behmann (1930) and Wood (1936), in which the patient is made to cough by the insertion of a swab or laryngeal mirror into the throat. The material so obtained can be swabbed over a slide and stained in the usual way, or better inoculated on to suitable culture media. It is doubtful, however, whether this method is as satisfactory as that of examining the stomach contents. According to Mishulow and her colleagues (1934), repeated examination of fæces is probably as satisfactory as that of gastric lavage specimens.

In pus the bacilli are often very difficult to demonstrate; Gardner (1926), following Spengler (1907), recommends the use of half-saturated aqueous picric acid as a counterstain.

The microscopical method of examination, though very simple, must not be undertaken without due regard to the errors of the technique employed. Microscopical examination alone cannot distinguish between tubercle bacilli and other types of acid-fast bacilli. Besides saprophytic acid-fast bacilli of the *Mycobacterium* group, which are widespread in hay, straw, dust, animal litter, soil, and water, there are a number of other organisms, such as the leprosy bacillus, Johne's bacillus, and some species of *Actinomyces*, which are acid-fast and which may occasionally give rise to confusion.

In order to avoid contamination of the material with these organisms, strict attention must be paid to the preparation of the actual films. In particular, all stains and reagents should be made up with fresh glass-distilled water; and all glass-ware should be soaked in 50 per cent. nitric acid for at least 15 minutes. Only new slides should be employed, since there is no simple method of removing acid-fast bacilli from slides on to which they have been fixed and stained, and every slide should be thoroughly flamed in a Bunsen burner immediately before use. If the material has to be rubbed up in a drop of water on the slide fresh glass-distilled water or water from a hot tap in constant use should be used, since acid-fast bacilli are usually abundant in cold-water taps (see Chapter 16). Care must be taken to avoid contamination of the cedar-wood oil reservoir from the surface of a previous film, and the oil-immersion objective should always be cleaned after examining a positive film. All apparatus, particularly if made of metal or rubber, used in the collection of catheter and other specimens, should be kept under conditions that will prevent the growth of saprophytic acid-fast bacilli on their surface (see A. H. Wells 1936).

When all these precautions have been taken, the importance to be attached to the finding of acid-fast bacilli will vary with the nature of the material examined. While acid-fast bacilli in sputum or cerebrospinal fluid may be regarded as almost certainly diagnostic of tuberculosis, their presence in fæces or urine must be interpreted with far greater caution, since the presence of saprophytic acid-fast, including smegma, bacilli cannot with certainty be excluded. In blood so many artefacts are liable to be present that it is extremely doubtful whether the search for acid-fast bacilli is justified at all.

Of recent years *fluorescence microscopy* has been used for the examination of tubercle bacilli. The method depends on the observation that tubercle bacilli, stained with a suitable dye and irradiated with ultra-violet light, transform the invisible short waves to longer visible rays. Hagemann (1937*a*, *b*), who introduced the method, at first advocated berberine sulphate as the dye, but later he (1938) recommended auramine. Observed through a yellow filter in the eyepiece, the fluorescent organisms appear as golden yellow rods against a violet background. The use of a dry 4 mm. objective with a × 10 eyepiece enables a wider field to be covered than with a 2 mm. oil-immersion lens. Numerous favourable reports have been published on the method (for references see Richards, Kline and Leach 1941, Bogen 1941, Lempert 1944), demonstrating its superiority over the Ziehl-Neelsen stain. It seems clear, however, that not all tubercle bacilli in a preparation are revealed by fluorescence microscopy, and that a higher proportion of positive results is obtained by cultural methods.

Cultural Methods in Diagnosis.—Increasing use is now being made of cultural methods in the diagnosis of tuberculosis. Uncontaminated material should be seeded directly on to a suitable medium. Contaminated material, such as sputum or gastric contents, should be first treated for 10–30 minutes with 15 per cent. antiformin, 10 per cent. HCl, 4 per cent. NaOH, or for 30–60 minutes with 5 per cent. oxalic acid, or some other agent capable of killing off non-acid-fast organ-

isms. Many different media have been recommended, particularly by American workers.

A. S. Griffith and F. Griffith (1907), working for the Royal Commission on Tuberculosis many years ago, made a careful comparison of whole egg, egg yolk, egg yolk agar, and various serum media, and found that the most satisfactory medium for *primary* cultivation was Dorset egg (3 parts egg to 1 part saline, sloped and inspissated at 75° C. for 2 hours on 2 successive days). Our own experience bears this out. Dorset's egg medium, which was prepared independently by A. S. Griffith and F. Griffith (1907), or a medium made up with 2–3 parts of egg yolk to 1 part of tap water (Corper and Cohn 1933*b*, 1942, Schwabacher 1937, McCarter and Kanne 1942), has proved superior to all the other media—Petroff, Petragnani, Löwenstein, Herrold, Corper and Uyei, Woolsey—commonly recommended. Many of these more complex media contain dyes, which have some inhibitory action on the growth of the organisms, and many contain glycerol, which has a selective action favouring the growth of the human tubercle bacillus at the expense of the bovine. The plain Dorset or egg yolk medium is free from these defects, and is equally suitable for the growth of both human and bovine types (see also Griffith, A. S., 1935).

Great care must be used to avoid contamination of the material with saprophytic acid-fast bacilli. Glass-stoppered vessels sterilized in the dry oven or the autoclave should be used. Rubber corks, and certainly bark corks, are to be avoided, since they may contain acid-fast bacilli. Even using the greatest precautions, it is impossible to avoid occasional contamination of the material with saprophytic acid-fast bacilli, and numerous workers have recorded the development of these organisms in otherwise sterile cultures (see Tiedemann 1931, Beaven and Bayne-Jones 1931, Pinner 1932, Cummins and Williams 1933, Griffith 1933, Bruynoghe and Adant 1933, Pinner 1935, Lester 1938). The fact that they usually are contaminants, and are not derived from the tissues, is evident from the fact that Schwabacher (1933) obtained them in cultures from normal rabbit blood.

Under favourable conditions the cultural method is more delicate than the microscopic method for the demonstration of acid-fast bacilli. Corper and Cohn (1933*a*) estimate that for the rapid microscopic detection of these bacilli in sputum, there must be about 100,000 per ml., whereas for their detection by the cultural method 10–100 organisms per ml. suffice. Some workers regard the cultural as more delicate even than the animal inoculation method (see Norton *et al.* 1932). The great drawback to the cultural method is that it provides no proof that the acid-fast bacilli which have been cultivated are really tubercle bacilli or are derived from the material under examination. No organism should, in fact, ever be reported as a tubercle bacillus until it has been finally identified by animal inoculation.

Of recent years, mainly owing to the assertion of Löwenstein that tubercle bacilli can be isolated from the blood of a high proportion of patients suffering not only from pulmonary and non-pulmonary tuberculosis, but also from such diseases as articular rheumatism, polyarthritis, chorea, multiple sclerosis, schizophrenia, and retro-bulbar neuritis, great attention has been paid to the subject of *tuberculous bacillæmia*. We do not propose to discuss this work in detail. Though tubercle bacilli may be demonstrated by cultural and animal inoculation methods in as many as 5–10 per cent. of severe, advanced and progressive cases of pulmonary tuberculosis, in 30–40 per cent. of cases of miliary and meningeal tuberculosis, and in 50 per cent. of fatal tuberculous cases examined post mortem, they can very rarely be found in the early stages of pulmonary or non-pulmonary tuberculosis. A search for these organisms in the blood is therefore of little value in diagnosis. The great majority of workers have entirely failed to substantiate Löwenstein's findings, which were almost certainly due to gross errors in technique. The whole subject has been critically reviewed by G. S. Wilson (1933). See also Report (1935*c*).

Serological Methods in Diagnosis.—Serological methods of diagnosis are not, on the whole, satisfactory.

Arloing and Courmont (for references see Wilson, G. S., 1925) in 1898 recommended the *agglutination test.* They obtained a homogeneous suspension of tubercle bacilli by growth of a special strain—now known to be of avian type (Wilson, G. S., 1925)—in glycerine broth. The reaction was considered positive when the organisms were agglutinated by the patient's or animal's serum in a dilution of 1–5 to 1–20. Positive results were obtained in 95 per cent. of early cases of pulmonary tuberculosis and in 50 per cent. of cases of surgical tuberculosis. The percentage, however, of normal persons and of patients suffering from non-tuberculous diseases who reacted positively was as high as 30–67 per cent. Numerous other workers (Mongour and Buard 1898, 1899, Beck and Rabinovitsch 1900, 1901, Bendix 1900, Neisser 1900, Koch 1901, Chang 1922, Courmont and Papacostas 1923, Spehl 1923, Karmann 1924) have reported on this test, and the general consensus of opinion is that as a method of diagnosis it is unreliable; many patients with tuberculosis fail to react, and many persons who appear perfectly well do react.

The *complement-fixation reaction* stands on a slightly firmer basis, though its value is still very much disputed. Numerous methods of performing the test have been devised, and still more numerous antigens. The French generally employ Calmette and Massol's technique with Besredka's antigen. Table 104, taken from Rieux (1926), gives some of the results that have been obtained.

TABLE 104

COMPLEMENT FIXATION IN TUBERCULOSIS.

Type of Disease.	Positive Results.
Pulmonary tuberculosis with positive sputum	85%
Clinically tuberculous pleurisy	46·6%
Tuberculous peritonitis	80%
Tuberculous meningitis	Rarely positive
Glandular tuberculosis	38%
Bone and joint tuberculosis	50%
Renal tuberculosis	90%
Healthy persons	10–15%
Persons suffering from non-tuberculous diseases	10%

Rieux concludes that every patient giving a strongly positive reaction, and who is not suffering from syphilis, leprosy, or malaria, is tuberculous. Besredka and Manoukhine (1914) conclude that in the first stage of tuberculosis the reaction is always positive; in the second stage it is generally positive; in the third stage it is often weakly positive or negative—especially before death. In this country the reaction is not held in such favour. Coulthard (1923) obtained the highest percentage of positive reactions in chronic pulmonary tuberculosis; he regards a strongly positive reaction as indicative of a relatively high degree of immunity, irrespective of the extent of the disease. In acute forms of tuberculosis, such as the miliary and acute pneumonic forms, and in tuberculous meningitis, the reaction was generally negative. In early pulmonary tuberculosis and in actively progressive pulmonary tuberculosis the results were disappointing. Sellers and Ramsbottom (1922) concluded that the test was of little value in diagnosis.

That the test is generally positive in chronic pulmonary tuberculosis there is little doubt; but these cases can generally be diagnosed by other means. In the early stages of the disease, when diagnosis by the usual methods is in doubt, the complement-fixation reaction is unreliable. It can at best be used only as a sub-

sidiary method, and even as such it must be interpreted with great reserve. (For further literature on the test, see Calmette and Massol 1914, Dudgeon, Meek and Weir 1914, Meek 1914, Radcliffe 1915, Urbain and Fried 1921, Brocq-Rousseu, Urbain and Cauchemez 1923, von Gutfeld and Weigert 1924, Douglas, S. R., and Meanwell 1925, Salomon and Valtis 1925, Valtis 1925, Wadsworth *et al.* 1930, Hünigen 1932, Rice *et al.* 1933, Urgoiti and Beato 1934, Hassegawa and Kochi 1939.)

The *precipitin reaction*, obtained by mixing the patient's serum with tuberculin, is of little or no practical importance. It is of interest to note that the antigen responsible for the complement-fixation and the precipitin reactions is a non-protein gum (Laidlaw and Dudley 1925, Mueller 1926).

Allergic Reactions in Diagnosis.—The tuberculin reaction may be performed in various ways; the tuberculin may be (1) rubbed on to the scarified skin—cutaneous reaction of von Pirquet (1907); (2) incorporated in an ointment and rubbed into the skin—percutaneous reaction of Moro; (3) injected intracutaneously—Mantoux's reaction (1910); (4) dropped into the conjunctiva—Calmette's ophthalmic reaction; (5) injected subcutaneously—Koch's original method (1890) (see p. 1328); (6) applied to the skin on a piece of thin filter paper impregnated with undiluted tuberculin and then dried—the patch test of Vollmer and Goldberger (1937, 1938, 1939*a*, *b*). Both the von Pirquet and the graded intradermal tests have already been described (pp. 1300–01). There is no question that the latter method is to be preferred. Nearly all persons with either a latent or an active tuberculous infection react positively to tuberculin, except when suffering from advanced disease, severe cachexia, or one of the infectious exanthemata. A positive reaction indicates the presence of a tuberculous focus in the body, but does not distinguish between an active and an inactive lesion. Except in the first few years of life a positive reaction is of very limited value in the diagnosis of clinical tuberculosis. On the other hand, a negative intracutaneous reaction, particularly to a 1/100 or 1/10 dilution, practically excludes the existence of tuberculous infection at any age (see Hart 1932). The Vollmer patch test has found some favour for use in infants and young children, but it is clearly neither as reliable nor as sensitive as the graded intradermal test (Report 1939, Kereszturi 1941).

The interpretation of different degrees of reactivity to tuberculin in terms of either diagnosis or prognosis (see von Gröer 1932, Johnston *et al.* 1934, Daniels 1944) is still open to doubt. As a rule children having a history of contact with tuberculosis, and patients suffering from active tuberculosis, have a very high sensitivity to tuberculin (Furcolow *et al.* 1941). For example, of 468 tuberculous patients investigated by Furcolow, Hewell and Nelson (1942), 99·6 per cent. reacted to 0·0001 mgm. or less of a particular batch of P.P.D., and some reacted even to 0·000000001 mgm. On the other hand, it was found that patients who were seriously ill, who had a short history or the pneumonic form of disease, and who were apparently resisting badly, had a lower degree of sensitivity.

It may be noted that in childhood a positive reaction may become negative with complete healing of the lesion, though the frequency with which this occurs is not known (see Lloyd and Macpherson 1933, Opie 1935, Zacks and Sartwell 1942). Dahlstrom's (1940) conclusion, however, that a high proportion of weak reactors in children become negative with the passage of years is very questionable; it has been criticized on the ground that the dilution of tuberculin he used was so low that many of the reactions he obtained were probably non-specific

(Furcolow *et al.* 1941). The allergic condition is often maintained by the persistence of tubercle bacilli in quiescent or healed lesions. According to Opie and Aronson (1927) tubercle bacilli are frequently detectable by guinea-pig inoculation even in apparently normal lung tissue, though Feldman and Baggenstoss (1938, 1939) and Saenz and Canetti (1938) doubt this. Howe (1938) has recorded rapid variations in the sensitivity of both tuberculous and non-tuberculous persons to tuberculins, associated to some extent with changes in blood pressure. It is generally assumed that a positive tuberculin reaction precedes or accompanies the appearance of recognizable lesions by X-ray examination, but Levine (1939) has brought evidence to show that in infants under one year of age the reverse sequence may be noted. This may perhaps be due to the poorer antibody response in early infancy than in later years. The comparative liability of tuberculin-positive and tuberculin-negative reactors to develop pulmonary tuberculosis will be considered later (pp. 1330–33).

A *delayed tuberculin reaction* has been described by Daniels (1943), developing several weeks or months after the intradermal injection of old tuberculin. It is regarded as an indication that the person has become infected with tuberculosis some time after the test. The reaction is apparently due to the persistence of tuberculin in the skin. If this interpretation is correct, the delayed reaction may serve as a useful indicator of primary infection.

Animal Inoculation Tests in Diagnosis.—The most delicate test for tubercle bacilli is animal injection, and the most suitable animal is the guinea-pig. The susceptibility of the guinea-pig is extremely high ; even minute amounts of infective material will render this animal tuberculous. The material—sputum, pus, milk, etc.—should be injected subcutaneously or intramuscularly into the thigh ; the advantage of intramuscular injection is that the local abscess which forms does not ulcerate through the skin. It is wise to inject at least two animals, in case one dies of secondary infection—an occurrence which is very common after the inoculation of urine or fæces. One animal should be killed 3–4 weeks later, and if no signs of tuberculosis are apparent, the other should be kept for 6–8 weeks after inoculation before being killed.

In positive cases killed at 6–8 weeks there will be found (1) a caseous local lesion ; (2) enlargement and caseation of the femoral, inguinal, sublumbar, portal, mediastinal, and cervical glands, the severity of the lesions diminishing as the distance from the site of inoculation increases ; (3) enlargement of the spleen with the presence of irregular necrotic areas ; (4) yellowish-green necrotic areas in the liver ; (5) a few discrete spherical tubercles in the lungs (for further details see p. 425). In animals killed after 3 or 4 weeks the lesions are less severe, and are restricted mainly to the lower portion of the chain of glands and to the spleen and liver. However experienced the worker may be, it is of paramount importance to make a microscopical examination for the presence of acid-fast bacilli. Direct smear preparations should be made from two or three enlarged lymphatic glands, as well as from the spleen, liver, and lungs, and a diagnosis of tuberculosis should not be made unless acid-fast bacilli are found in the internal organs or in glands not directly draining the site of inoculation. The reason for this is that saprophytic acid-fast bacilli may be present in the local lesion and the regional lymphatic glands, though they are usually incapable of penetrating further into the tissues after inoculation by the intramuscular route. It is dangerous to make a diagnosis of tuberculosis unless at least one gland shows caseation. In all doubtful cases cultures should be put up on ordinary media for the diagnosis of *Salmonella*, *Brucella*, *Pasteurella*, and pyogenic infections, and on to suitable media for the growth of tubercle and saprophytic acid-fast bacilli. Whenever necessary, a fresh animal should be inoculated intramuscularly with some ground-up pathological

material, and with any acid-fast bacillus that may develop in culture. Special care must be taken in the microscopic examination of material to guard against the presence of acid-fast bacilli derived from water or from tuberculous tissue on the instruments used for making a post-mortem examination, since boiling does not destroy their property of acid-fastness. Heating the instruments in a Bunsen flame to dull redness usually suffices for this purpose.

The tuberculin test, made by inoculating 0·1 ml. of a 1/50 to 1/100 dilution of old tuberculin into the skin of the flank, may be used for the diagnosis of tuberculosis in a guinea-pig during life (see p. 1328). It is of particular value in furnishing a guide to the time at which the animal should be killed. The test is usually very reliable, provided no reaction is considered positive unless the local lesion reaches 10 mm. in diameter. Tuberculosis, however, should never be diagnosed on a tuberculin test alone; the result must always be confirmed by post-mortem examination.

IMMUNITY IN TUBERCULOSIS

Our knowledge of the defensive mechanism of the animal body against the tubercle bacillus is very incomplete. A study of the humoral antibodies—notably agglutinins, complement-fixing bodies, and opsonins—has failed to throw much light on the subject; there is apparently no strict relationship between the antibody content of the person's serum and his resistance to tuberculosis. Most of the knowledge that we have gained consists of isolated observations, which it is impossible as yet to piece together except in the form of a purely tentative hypothesis. A summary of these observations must necessarily be disjointed.

Koch's Phenomenon.—If a healthy guinea-pig is injected subcutaneously with a pure culture of tubercle bacilli, the slight local lesion caused by the injection heals in a few days; in 10 to 14 days, however, a hard nodule appears, which breaks down and remains as an ulcerating sore till the animal dies; the regional lymphatic glands swell considerably and undergo caseation. But if a tuberculous guinea-pig—one that has been injected 4 to 6 weeks previously—is injected subcutaneously into the opposite thigh with a pure culture of tubercle bacilli, the slight local lesion heals rapidly, but no nodule forms. Instead, on the 1st or 2nd day after inoculation, a circular dark-coloured area of induration, about ½ to 1 cm. in diameter, appears at the site of injection. The next day the skin over this ecchymotic area undergoes necrosis; and later it is thrown off leaving a shallow ulcer, which heals quickly; the focal lymphatic glands remain unaffected. This reaction was first observed by Koch (1891*a*), and is known as Koch's phenomenon. For its success three conditions are necessary (von Löwenstein 1913): (1) the primary infection must be a slight one, so that the disease runs a chronic course; (2) the re-infecting dose must be given as late as possible after the sensitizing dose; the longer the animal has been infected the higher is its resistance; (3) the re-infecting dose must not be too large.

It is clear that the reaction of a tuberculous guinea-pig to a fresh infection is different from that of a normal guinea-pig to a primary infection. Some experiments conducted by Debré and Bonnet (1922) will make this clearer.

A. One group of guinea-pigs was injected with 0·1 mgm. of tubercle bacilli every day for 5 days. *Result.*—Five nodules appeared, identical in their period of incubation (8 to 10 days), in their evolution, and in their accompanying glandular reaction. *B*. Another group of guinea-pigs was injected with 0·1 mgm. of tubercle bacilli every 5 days on five

successive occasions. *Result.*—The first two nodules and their accompanying glandular reaction were identical with those in Series *A*. The third nodule appeared after a shorter incubation period than the first two, was much smaller—the size of a lentil instead of a nut—did not ulcerate, and caused only a slight glandular swelling. The fourth and fifth nodules were identical with the third. *C*. Another group of guinea-pigs was injected with 0·1 mgm. of tubercle bacilli every 10 days on five successive occasions. *Result.*—The first nodule and its accompanying glandular reaction were similar to those in Series *A*. The second and third nodules resembled the 3rd, 4th, and 5th nodules of Series *B*. The fourth nodule produced either a typical Koch's phenomenon, or rapid abscess formation followed by cicatrization, without any glandular enlargement. The fifth nodule constantly provoked the typical eschar described by Koch. With these particular doses, it will be seen that Koch's phenomenon did not appear till about 6 weeks after the first injection. The main lesson to be learnt from this experiment is that the "chronical" supersensitization of a tuberculous guinea-pig is impracticable after the appearance of the local abscess. Before the local lesion has appeared the animal reacts to a fresh infection just as a normal animal; but once the local lesion due to the first injection has developed—generally in 8 to 10 days—the lesions produced by subsequent injections become less and less, till after 6 weeks the freshly injected organisms call forth an acute reaction, quite unlike the chronic process that follows a primary infection.

These observations are similar to those made on experimental syphilis, which have shown that it is possible to produce in the ape a series of chancres, so long as the second injections are made before the appearance of the primary chancre; once the primary chancre has appeared, further inoculation of syphilitic material fails to produce a fresh chancre. The guinea-pig that has developed a local tuberculous lesion, or the ape that has developed a primary chancre, reacts differently from a normal animal to a fresh infection. It is, so to speak, at once more sensitive and more resistant to the infective material than the normal animal. This state of hypersensitiveness is generally known as *allergy*. Whether the immunity is only a symptom-immunity, or whether it is sufficiently high to prevent the actual invasion of the organisms at the site of the second injection is not known, but the experiments of Thomsen and Pedersen-Bjergaard (1933) suggest that it may be of the latter type.

Koch (1891*a*) found that his phenomenon could be reproduced even when dead tubercle bacilli were employed for the second injection. Killed suspensions of tubercle bacilli, when injected subcutaneously into normal guinea-pigs, give rise merely to a local sterile abscess; there is no constitutional disturbance whatever. But if they are injected into a tuberculous guinea-pig, one of two things happens, depending on the dose: if a large dose is used the guinea-pig dies in 6 to 48 hours; if a smaller dose is used, there is a local necrosis of the skin—Koch's phenomenon. The allergic guinea-pig, in fact, responds to the injection of dead tubercle bacilli in very much the same way as to that of living bacilli. Koch therefore argued that the reaction must in each case be due to some chemical substance present alike in living and dead bacilli. This active principle he endeavoured to obtain in a soluble form (Koch 1891*b*).

Tuberculin : Preparation, Purification, and Standardization.—Koch grew tubercle bacilli for 6 to 8 weeks at 38° C. in flat-bottomed flasks containing 5 per cent. glycerine broth. The culture was then placed in a water-bath at 100° C., evaporated to 1/10 of its volume, and freed from bacilli by filtration through a clay or kieselguhr candle. The resulting filtrate was a clear, brown, syrupy fluid; as it contained

40–50 per cent. of glycerine, no antiseptic was required for its preservation. It is generally known as Koch's *old* or *original tuberculin*, and contains all the substances in the culture medium, including those formed from the medium by the bacilli, and the disintegration products of the bacilli themselves, not precipitated by 50 per cent. glycerine. This old tuberculin was found to contain in an impure form the toxic constituents that Koch had been looking for. It is heat-stable, and, when undiluted, will resist autoclaving for half an hour.

Later, owing to the failure of old tuberculin in the treatment of tuberculosis, Koch (1897) prepared a series of tuberculins in the hope of obtaining one that should be immunizing without being toxic. These, which were called Tuberculin A, Tuberculin O, Tuberculin R, and Tuberculin BE, are now mainly of historic interest (for their preparation see Topley and Wilson, 2nd edition, p. 1041).

Of late years attention has centred on the preparation of a product as free as possible from substances capable of giving rise to non-specific reactions when used for graded intradermal tuberculin tests. For this purpose synthetic media have replaced broth (Long and Seibert 1926, Douglas and Hartley 1934*b*). From the old tuberculin thus produced a substance known as *purified protein derivative* (P.P.D.), containing the active principle of tuberculin, has been isolated and purified by ultrafiltration to get rid of the salts and glycerol, followed by precipitation with trichloracetic acid (Seibert *et al.* 1934). (See also Gough 1934.) Further work has resulted in the separation of a protein derivative with a molecular weight of only 10,500, containing very little nucleic acid and polysaccharide, and having twice the potency of the previous product (Seibert, Pedersen and Tiselius 1938, Seibert 1941*a*, *b*, Seibert and Glenn 1941). Though P.P.D. constitutes an advance on O.T., it is still far from perfect as a specific diagnostic agent. Successive preparations differ in potency, in specificity, and in antigenicity (McCarter and Watson 1942), so that standardization is just as necessary as with O.T. (For the chemistry of tuberculin, see Seibert 1944.)

Old tuberculin is most conveniently standardized against a standard tuberculin —hitherto issued by the Frankfurt Institute but now stored at the State Serum Institute, Copenhagen (see Report 1931*a*)—using the intradermal inoculation of sensitized guinea-pigs (see Okell and Parish 1927, Parish 1938).

This method, originally described by Römer (1909), consists in inoculating the tuberculin into the animal's flank. As many as 12 or 16 injections can be made on the depilated skin of the same animal. Twenty-four hours after injection a large papule is seen with a hæmorrhagic necrotic centre—the so-called cockade reaction. The reaction reaches its height after 48 hours, and later necrosis becomes more marked. Slight infiltration and swelling cannot be regarded as positive. Römer's test is positive about 3 weeks after infection. Dilutions of the tuberculin to be tested are compared with the standard tuberculin, and the relative potencies of the two products determined.

Tuberculo-protein preparations are standardized against old tuberculin, either by using the guinea-pig method or by making titrations on human subjects (see Lichtenstein 1934, Appel *et al.* 1934, Barnwell and Pollard 1934, Aronson 1934, Long 1934, Long *et al.* 1934).

The Reaction to Tuberculin.—Koch found that 2 ml. of old tuberculin injected subcutaneously into a normal guinea-pig had very little effect on it; but the injection of as small a quantity as 0·01 ml. into a tuberculous guinea-pig—infected 8 to 10 weeks previously—killed it within a few hours. Post mortem, the local lesion at the site of the tuberculin injection was very congested, and often dark or

almost violet in colour: the focal glands were also very congested: the liver and spleen, besides their tuberculous lesions, showed on the surface numerous punctiform dark red spots looking like ecchymoses, but which microscopically were found to consist of enormously distended capillaries in the neighbourhood of the tuberculous foci, filled with red blood corpuscles. That is to say, the effect of the tuberculin was to produce (1) a local lesion, at the site of injection: (2) a focal lesion, around the tubercles in the tissues: and (3) a constitutional reaction, terminating in death (see also p. 1161).

Analogous observations were made on man. A new-born infant, which has never come into contact with tuberculosis, can withstand the injection of 1 ml. of old tuberculin. A healthy adult, who has been infected, but who is not suffering from clinical tuberculosis, can withstand the injection of about 0·01 ml. without suffering from more than transient malaise and slight pains in the limbs. But the same quantity injected into a clinically tuberculous patient gives rise to a severe reaction, characterized by malaise, pains in the limbs, cough, dyspnœa, rigor, vomiting, and a high fever: the temperature begins to rise about 4 hours after the injection, reaches a maximum of 102° to 104° F., and passes off in 12 to 15 hours. As in the guinea-pig, the reaction is (1) local—an inflammatory reaction occurs at the site of injection (*Stichreaktion*): (2) focal—acute congestion occurs around tuberculous foci; this can be actually observed in lupus patients; an injection causes redness and swelling of the lesion, lasting for 2 or 3 days, followed by the formation of crusts of dried exuded serum, which drop off in 2 or 3 weeks leaving a clean red scar: (3) a constitutional reaction, already described.

The tuberculin reaction can be obtained not only in animals actually infected with the tubercle bacillus, but also in animals that have been injected with dead tubercle bacilli. Bessau (1916), for example, was able by the intraperitoneal injection of 1 mgm. of human tubercle bacilli killed by heat at 65° C. for 2 hours, or by the subcutaneous injection of 5–15 mgm., to produce a skin sensitivity to tuberculin in guinea-pigs; the tuberculin reaction was often as strong as in tuberculous animals. But this hypersensitiveness did not develop immediately; it took as a rule 2 to 3 weeks, and sometimes as long as 4 months, to develop. These observations have been frequently confirmed (Nakayama 1924, Zinsser and Petroff 1924, Lange and Freund 1926).

Unlike dead bacilli, old tuberculin, even after a long course of injections, usually fails to sensitize the guinea-pig (but see Aronson and Nicholas 1933). According to Seibert (1932), this is due partly to the fact that the dosage is inadequate, and partly to a diminution in the antigenic activity of the protein in old tuberculin as the result of the heat used in its preparation. Tuberculo-protein, on the other hand, if given in a total dosage of about 30 mgm., corresponding to about 60 ml. of pure tuberculin, is capable of bringing about typical skin sensitiveness in the guinea-pig. Rabbits can also be sensitized if larger doses are used, but the tuberculin reaction in the rabbit is less constant than in the guinea-pig, and not all tuberculous rabbits react to tuberculin (see Report 1913). It is very difficult to regard Seibert's explanation as wholly satisfactory, as she herself realizes. Why is it, for example, that guinea-pigs can be sensitized to tuberculin by a single dose of 0·1–0·5 mgm. of dead tubercle bacilli (see Tytler 1930, Douglas and Hartley 1934*a*), when it requires as much as 30 mgm. of purified protein to produce the same effect?

Bovine and human tuberculin appear to be similar in their toxic effects (Wolbach and Ernst 1904). Moreover, tuberculins prepared from strains differing in virulence have apparently the same potency (see Seibert and Morley 1933). There is evidence,

however, that human and bovine tuberculins are not identical; a difference between them can be detected by means of cross-anaphylactic reactions in the guinea-pig (see Lewis and Seibert 1931.)

Since dead bacilli are able to sensitize animals to tuberculin, some sensitizing substance, tuberculin or tuberculin-like, must be liberated from the bacilli in the tissues. These substances sensitize the cells of the body, which are then able to react more strongly to the subsequent injection of tuberculin.

The generalized tuberculin reaction has been held to differ from anaphylaxis in that (1) the passive transference of tuberculin sensitivity from an infected to a normal animal has never yet been satisfactorily demonstrated: (2) the focal reaction, which occurs in the immediate neighbourhood of foci of active tuberculous infection after the injection of tuberculin, has no counterpart in anaphylactic shock: (3) the reaction to tuberculin is delayed for some hours; in anaphylaxis the shock occurs directly after the injection: (4) after tuberculin there is a rise in temperature; in anaphylactic shock there is a fall in temperature (see Chapter 51).

It is doubtful whether much attention should be paid to these differences. Lewis and Seibert (1931), for example, have apparently been able to produce fatal anaphylactic shock in guinea-pigs by the use of tuberculo-protein in place of tuberculin (see also Enders 1929). The sensitizing dose was 0·1–15·0 mgm. and was given intraperitoneally; the "shocking" dose was 5–15 mgm. and was given intravenously. Similar shock could be produced by the intravenous injection of tuberculo-protein into tuberculous guinea-pigs. Passive sensitization was also successfully accomplished by the intravenous injection of guinea-pigs with the serum of other guinea-pigs that had been actively immunized by repeated inoculation with tuberculo-protein. Twenty-four hours after the receipt of the serum, the passively sensitized animals were inoculated with 10–15 mgm. of tuberculo-protein. Most of them died of shock within 5 minutes. The serum of tuberculous guinea-pigs, however, was unable to convey passive sensitization to normal animals. Lewis and Seibert believe that bacterial protein preparations are usually bad anaphylactogens, because in their native state they are very insoluble, and when brought into solution they are either denatured, altered, or hydrolysed, particularly if they are exposed to heat, cold, sunlight, acids, or alkalies. Ultra-filtration and precipitation with ammonium sulphate are free from these defects, and were used for the preparation of the tuberculo-proteins in the experiments just described. Most workers have used old tuberculin, which has been submitted to prolonged heating, and it is probable that their failure to obtain anaphylactic shock may be attributable to this cause. On the whole it seems likely that the differences between the generalized tuberculin reaction and the typical anaphylactic reaction are dependent far more on solubility and rapidity of absorption of the protein than on any fundamental differences in the nature of the two reactions. In this connection it is interesting to note that Brahic and Veyron (1939) found a striking parallelism between the reaction to the intradermal inoculation of tuberculin and that to histamine (see p. 1148). Patients giving a strong tuberculin reaction likewise reacted strongly to histamine, and those with a weak tuberculin reaction gave only a feeble histamine reaction.

The Relation between Allergy and Immunity

Of recent years, owing partly to the observations of Heimbeck and his Scandinavian colleagues on the development of tuberculosis in nurses and students, and partly to the observations on animals of Rich and his colleagues in the United States, the relation between allergy and immunity has been under active discussion. Two opposing views have been expressed, each based on circumstantial evidence. It must be our endeavour to present the two sides of the case as fairly as we can.

In the first place we must define our terms, because some confusion has arisen from lack of clarity in this respect. Rich, for example, defines allergy and immunity mainly in terms of results. If allergy is defined as a state in which the tissues are damaged and immunity as a state in which they are protected, then clearly allergy must be bad and immunity good. In so far as bacteriology is concerned, we would rather define allergy as a condition which may develop as the result of introducing bacteria or their products into the body, and which manifests itself as a heightened or accelerated inflammatory response towards a particular type of antigen or other related substance, irrespective of the balance of harm or benefit that the altered response confers on the allergic host. Acquired bacterial immunity may be defined, for our purpose, as a state of resistance of the host, partial or complete, specific or non-specific, towards a given bacterium or its products. The problem is to determine whether allergy, as just defined, plays any part in the resistance of the host to tuberculosis.

Observations on Human Beings.—Stimulated by Scheel's observations at the Ullevaal Communal Hospital at Oslo on the frequency of tuberculosis in probationer nurses, Heimbeck (1927, 1932, 1933, 1936) tested with tuberculin every nurse entering the hospital. The training course lasted three years and comprised a period of service in the tuberculosis wards. Of 905 probationers submitted to the von Pirquet test, 625 gave a positive and 280 a negative reaction. The tests were begun on January 1st, 1924, and up to the time of publication in 1936, 27 of the initially tuberculin-positive and 96 of the initially tuberculin-negative nurses are said to have developed tuberculosis; in the former group none, and in the latter group 10, died. Thus the incidence of tuberculosis was about 8 times as high in the negative as in the positive group.

From 1927 onwards Heimbeck endeavoured to protect the von Pirquet negative nurses by vaccinating them subcutaneously with B.C.G. (see p. 1337). As the result of this procedure many of the nurses developed a positive tuberculin reaction, but some did not. The two groups were followed up, and it was found that of 287 nurses who became von Pirquet positive, only 8 developed tuberculosis, whereas of 107 who remained negative, no fewer than 22 developed tuberculosis. The incidence of tuberculosis was therefore over 7 times as high in the negative as in the positive group.

From these observations Heimbeck concludes that allergy is an expression of immunity. Before accepting, however, these figures as conclusive, two points must be noted. Firstly, instead of following up initially positive and initially negative nurses over the same length of time, Heimbeck transferred in his analysis the initially tuberculin-negative nurses to the positive group within one year of their primary infection. The result of this was that if the nurse developed symptoms of tuberculosis within a year of her primary infection, she appeared among the initially negative group that became tuberculous. If, on the other hand, she remained perfectly well for a year after becoming von Pirquet positive, she went to swell the group of initially positive nurses who failed to develop tuberculosis. Since Israel, Hetherington and Ord (1941) found that the majority of tuberculous lesions in initially tuberculin-negative nurses occurred during the year following the appearance of a positive reaction, there seems little doubt that the effect of this transfer must have been to increase the disparity between the two groups and weight the odds in favour of the initially tuberculin-positive group. In the second place, the term tuberculosis comprised pleurisy, pulmonary infil-

tration, erythema nodosum, and glandular tuberculosis as well as manifest pulmonary tuberculosis. If we restrict ourselves to this last group of cases, with tubercle bacilli in the sputum, then we find that only 4 out of 27 patients in the initially tuberculin-positive group and only 13 out of 96 in the initially negative group who are said to have developed tuberculosis suffered from manifest pulmonary disease. This makes little difference to the comparison between the two groups, but it does give a very different picture of the incidence of tuberculosis in the Oslo nurses.

Observations similar to those of Heimbeck have been made by other workers on nurses and medical students. Those recorded by Kristenson (1933) and Scheel (1935) in Scandinavia, Rist (1939) in Paris, and Israel, Hetherington and Ord (1941), Brahdy (1941), and Zacks and Sartwell (1942) in the United States are in general consonance with the results obtained by Heimbeck, though the differences between the tuberculin-positive and tuberculin-negative groups were usually considerably less than those recorded by Heimbeck. On the other hand, Jacobson (1933) in Denmark, and Plunkett, Weber, Siegal and Donk (1940) in the Newark State school for mental defectives found little difference between the two groups; and Shipman and Davis (1933) in California and Würtzen and Sjørslev (1936) recorded results that were actually in favour of the tuberculin-negative group (see also Ljung 1939).

We may turn now to investigations carried out under very different conditions, namely among the mine labourers on the Witwatersrand (Report 1932*b*). These labourers, or "boys," came from native territories in which tuberculosis was endemic. On arrival at the mine, they were submitted to a cursory physical examination in order to eliminate those with obvious disease, and were tested intradermally with 0·1 ml. of a 1/5000 dilution of old tuberculin. Their after-history is summarized in Table 105.

TABLE 105

SHOWING PROPORTION OF POSITIVE AND NEGATIVE REACTORS TO 1/5000 TUBERCULIN DEVELOPING CLINICAL TUBERCULOSIS. (S. African gold miners.)

Type of Reaction.	Number.	No. developing Tuberculosis.	Incidence of Tuberculosis per 100,000.
(*a*) Negative	32,864	114	347
(*b*) Weakly or moderately positive .	57,236	391	683
(*c*) Strongly positive.	3,879	60	1,547
(*b* and *c*) Positive	61,115	452	738

It will be seen that the incidence of tuberculosis was more than twice as high in the positive as in the negative reactors, and that the stronger the reaction was to tuberculin, the greater was the liability to clinical tuberculosis.

Before concluding that these figures are in direct contradiction to those of Heimbeck, it is important to realize that the two populations concerned differed in several respects. Heimbeck's nurses had been physically examined before being enrolled as probationers and all those with a history or with symptoms of tuberculosis had been excluded. The South African "boys" had undergone a much less rigorous inspection. As a result, probably a number of the positive reactors were suffering from latent active lesions, which progressed under the severe physical exercise in the mines to manifest pulmonary tuberculosis. This interpretation is

borne out by the fact that one-third of the strongly positive reactors broke down within three months of their admission to the mines. It may be noted that, though the incidence of tuberculosis was higher in the positive than in the negative group of Rand labourers, the severity of the disease and the case-mortality rate were both considerably greater among the initially negative reactors. The lesions showed little or no tendency to fibrosis, and the disease became rapidly generalized, whereas in the positive reactors fibrosis occurred and the lesions often remained confined to the lungs. Thus, in 24 per cent. of tuberculosis cases in the negative group the disease ran a septicæmic course, as opposed to 4 per cent. in the strongly, and 5 per cent. in the moderately or weakly, positive tuberculin reactors.

Two more discrepant observations may be mentioned. Myers and Hermann (1934) in the United States found that children who were tuberculin-positive while at school developed tuberculosis during the next 10 or 12 years far more frequently than their tuberculin-negative classmates. Similar findings were recorded by Pope, Sartwell and Zacks (1939). Yet Zacks and Sartwell (1942) found that in a school for the feeble-minded the proportion of non-reactors who developed tuberculosis during a period of 10 years was 4 times as great as that of initially positive reactors. Finally, Levine (1941) found that in children under 6 years of age the mortality rate following exogenous infection was much the same in those who were previously tuberculin-positive as in those who were previously tuberculin-negative, suggesting that sensitization of the tissues did not render a subsequent infection either more or less dangerous.

We have quoted enough evidence to show that there can be no simple relationship between allergy and immunity in the human subject. Under one set of conditions the advantage is on the side of the allergic, under another set on the side of the non-allergic. Admittedly some of the data are not free from criticism. Among school children, for example, it is probable that those who react to tuberculin are more likely to be exposed to further infection in the future than those who fail to react and are probably living in homes free from infection. Nevertheless the recorded differences between the behaviour of tuberculin-positive and tuberculin-negative reactors are too great to be explained away by errors of this sort.

On the whole the evidence suggests that persons who have passed safely through their primary infection are less liable to develop manifest tuberculosis when exposed to fresh infection than are those with no previous experience of the tubercle bacillus. The degree of benefit they derive, however, from their allergic state is probably dependent on a number of factors of which we have only imperfect knowledge. On the other hand, when they are exposed to unfavourable environmental conditions, such as overwork, undue fatigue, inadequate nutrition, and insufficient sleep, in the absence of any unusual exposure to new infection, allergic patients are more liable to break down through endogenous infection than those who are non-allergic. Put more succinctly, in the heavily infected *milieu* of a tuberculosis hospital the advantage is with the allergic; in the less heavily infected but physically strenuous conditions of the mine or the factory, the advantage is with the non-allergic.

Observations on Animals.—Turning to the experimental study of allergy, we may start with the observations of Rich (1929, 1930, 1931, 1933*a*, *b*) and of his colleagues (Rich and McCordock 1929, Rich and Brown 1930, Rich, Chesney and Turner 1933, Rich, Jennings and Downing 1933, Rothschild *et al.* 1934).

Briefly, it was found that guinea-pigs which had been submitted to a mild tuberculous

infection and then desensitized by repeated massive doses of tuberculin were just as resistant to superinfection with virulent tubercle bacilli as control animals that had received the same primary infection but had not been desensitized. From this observation Rich concludes that allergy plays no part in immunity.

Numerous workers (for references see Wilson, Schwabacher and Maier 1940) have confirmed Rich's fundamental observation, but not all would agree with him in its interpretation. A great deal hangs on the meaning of allergy and of desensitization. Is it possible, for instance, to desensitize a tuberculous guinea-pig so completely that it can be regarded as non-allergic ? The experimental evidence seems to show that, though the skin reactivity may be practically, though only temporarily, abolished by massive doses of tuberculin, it is almost impossible to achieve and maintain this state without seriously impairing the health and life of the animals. It is therefore doubtful whether a true comparison has ever been made between completely desensitized and control animals.

Again it is to be questioned whether desensitization of the skin necessarily implies desensitization of other tissues. Freund (1929), for example, showed that young tuberculous guinea-pigs were just as sensitive as adult guinea-pigs to the intraperitoneal inoculation of tuberculin, even though they were practically insensitive to its inoculation intracutaneously ; and Hehre and Freund (1939) found that tuberculous rats, though developing no local inflammatory reaction after injection of tuberculin into the skin, nevertheless died of tuberculin shock.

Another difficulty is to know whether the liberal daily dosage with tuberculin necessary to maintain the desensitization of the guinea-pig's skin has any immunizing effect. Rich assumes that it has not. Experimental evidence, it is true, suggests that injection of tuberculin leads to no increase in resistance of the *normal* animal, but that in *allergic* animals it sets up a series of focal reactions which may confer on the tissues some degree of resistance to the invasion of tubercle bacilli.

The work of Lurie (1934) indicates that the destruction of avirulent tubercle bacilli which occurs in the internal organs is greatest when the maximum degree of hypersensitivity is reached. The observations of Freund and Opie (1938) suggest that, though there is no linear relationship between skin sensitivity and resistance to the intravenous injection of virulent tubercle bacilli, nevertheless animals that are moderately allergic at the time of infection usually prove most resistant. Our own observations (Wilson *et al.* 1940) point in the same direction. Thus we found that a moderate degree of allergy in vaccinated animals at the time of infection was more beneficial than a low or high degree, and that in both normal and vaccinated animals the survival time tended to be more or less proportional to the degree of skin sensitivity reached after infection. In this connection the observations of Lurie (1938, 1944*b*) are of special interest. Lurie found that in genetically resistant rabbits the skin was less permeable to Indian ink, and that skin sensitivity to tuberculin was more rapidly established after infection than in genetically susceptible animals. In other words, animals capable of developing the greatest degree of allergy after infection, and presumably of restricting the invasion of the bacilli, tended to have the greatest resistance. The corollary of this, namely that bacilli multiply most in the tissues of animals having a very low degree of skin sensitivity, has been reported by Woodruff and Kelly (1942).

Many years ago Krause (1925) and Willis (1925) showed that the dissemination of tubercle bacilli from a local cutaneous or subcutaneous infection was very much slower in the allergic than in the normal animal. The local fixation of tubercle bacilli may depend, as Rich appears to believe, on the presence of agglutinating antibodies ; but since carbon particles (Lurie 1936) and dye particles (Joyner and Sabin 1938) are similarly fixed, it is probable that a non-specific cellular mechanism is at work as well. The observations of Freund and Angevine (1938) indicate that tubercle bacilli may multiply at the site of fixation, and that the retardation of lymphatic invasion is therefore probably due to mechanical factors (see Chapter 47). The mechanism of fixation may be reinforced

by the presence of specific antibodies, which tend to accumulate at the site of inflammatory areas in allergic animals (Menkin 1939, Fox 1936).

Another characteristic which, according to Lurie (1939*a*, *b*, 1942), is more closely related to immunity than anything else, is the increased phagocytic power of the mononuclear cells in tuberculous animals. This is manifested both *in vitro* and *in vivo*, and appears to be independent of humoral antibodies. The mononuclear cells accumulate more rapidly at the local site of infection in vaccinated or tuberculous animals than in normal animals, and destroy the ingested tubercle bacilli more completely. How far this cellular sensitization may be regarded as part of the allergic response may perhaps be questioned, but we find it difficult to separate it from the heightened or accelerated inflammatory response that we regard as characteristic of the allergic state.

All of this evidence is compatible with the thesis that an accelerated response of the tissue cells to the tubercle bacillus or its products is of survival value to the host. This conclusion agrees with that reached by Menkin (1938) in his review on the rôle of inflammation in immunity. The beneficial effect of desensitization with tuberculin seems to us to lie not in abolishing allergy, but in converting a high into a low degree of allergy. The repeated doses of tuberculin set up minor inflammatory reactions around each tuberculous focus in the body, and these tend to retard dissemination of the tubercle bacilli and to prevent the rapid and destructive caseation in the internal organs which seems more prone to occur in very hypersensitive animals. It seems clear, however, that allergy is not the only mechanism concerned in immunity. Under the section on Heredity for instance, we have already referred to Lurie's (1941) observations on rabbits, in which he found that genetically resistant rabbits, besides developing a rapid and intense degree of allergy, were distinguished from susceptible animals by a low skin permeability and the power to form antibodies quickly and in high concentration.

Summarizing, we believe that the experimental and the field evidence suggest that immunity to tuberculosis is composite, and that allergy is one of the factors which often, though not always, contributes to its establishment. The fixation of the organisms that occurs at the site of infection in the allergic subject prevents rapid lymphatic dissemination and enables the sensitized mononuclear cells to ingest and destroy the invading tubercle bacilli more quickly than in the non-allergic subject. On the other hand, there is some evidence that in the internal organs the violence of the inflammatory reaction that occurs around tuberculous foci in the highly allergic subject may lead to such rapid tissue necrosis that the resulting caseous abscess may constitute a source of general toxæmia, or by rupturing into a bronchus, for example, may pave the way for secondary infection. In general, allergic subjects are probably less liable to develop clinical tuberculosis than those who have never been in contact with the tubercle bacillus, but there are numerous exceptions. Allergic persons, with latent active lesions, particularly if exposed to unfavourable environmental conditions, are sometimes more liable to develop clinical manifestations of disease than those who are completely non-allergic. In a highly infective environment, however, as in the tuberculosis wards of hospitals, the non-allergic are usually at a disadvantage. The relationship between allergy and the liability to develop tuberculosis is complex, and depends on a number of factors, of which the state of the lesions, the risk of exposure to infection, and the environmental conditions are probably among the most important (for general discussion on relation of allergy to immunity see Chapter 51).

Our knowledge of immunity to tuberculosis is very deficient. There is evidence to suggest that genetic resistance plays as important a part as the development of acquired immunity. Neither type is absolute; both are subject to fluctuations; and acquired immunity, in particular, rarely seems to be of a high order or of long

duration. The logical development of these conclusions will be dealt with in the next section.

THE PROPHYLAXIS AND TREATMENT OF TUBERCULOSIS

Prevention of Infection.—General hygienic measures include the prevention of spitting, except into a suitable receptacle, which can be sterilized or burnt; the prevention of coughing, except into a handkerchief; the segregation of advanced cases of open tuberculosis; the prevention of overcrowding; the protection of infants and children from contact with tuberculous parents; the pasteurization or boiling of all milk except that coming from tubercle-free herds; and education in personal hygiene and in diet (see Discussion 1935, Hart 1937). Whether the risk of infection indoors can be reduced by ultra-violet irradiation of the atmosphere, it is as yet too early to say; but Lurie (1944*a*), working with rabbits, has reported some success by this means.

In view of the conclusions reached in the last section we believe that avoidance of exposure to massive and repeated infection and unfavourable environmental conditions is of greater importance than the attempt to develop an acquired immunity. There has in the past been a great tendency to overlook the fact that tuberculosis is an infectious disease. Little or no attempt has been made to protect nurses, medical students, and doctors against the unduly high risk of infection that practice of their profession entails, with the result that the incidence of tuberculosis has been unnecessarily high among these classes of the population. Again, too rigid an interpretation of Koch's phenomenon has led to the conclusion that tuberculous patients cannot be re-infected, regardless of the possible danger that a highly allergic patient may incur from an acute local inflammatory disturbance of a pulmonary focus resulting from re-infection. Exposure to any disease, even those in which recovery is followed by a high degree of immunity, is always accompanied by danger, and is not justified except in such an instance as measles in which an attenuated attack can be ensured by appropriate serum treatment. The avoidance of unnecessary exposure to infection is particularly desirable in the case that we are now considering, where infection never seems to be followed by a high degree of immunity, and is sometimes followed by clinical disease from which complete recovery is uncommon.

The argument is sometimes advanced that tuberculous milk is not an unmixed evil. The tubercle bacilli that it contains are presumed to cause mild infections in infants and children which serve to protect them against subsequent infection with the human bacillus. This argument cannot be too strongly condemned. The bovine tubercle bacillus is no less virulent than the human (see Griffith 1925*b*), and many of the infections to which it gives rise are severe and fatal. The argument involves the assumption that the exposed population, as a whole, benefits from the development of an acquired immunity, and ignores altogether the process played by natural selection in weeding out the least resistant members of the population. Even if it were true, the price to be paid, in terms of lives and suffering, for any such advantage is far too high (see p. 1317 and Griffith 1925*b*, Report 1934). If acquired immunity is to be developed, let us at least endeavour to produce it in some more intelligent way, less fraught with danger of active disease and death.

Prophylactic Vaccination against Tuberculosis.—Numerous attempts have been made to vaccinate animals against tuberculosis. Koch (1897), using tuberculin R.,

stated that by giving large doses, about 2–3 mgm., he was able to immunize a considerable number of guinea-pigs, so that they withstood repeated injections of virulent tubercle bacilli without becoming infected. These results, however, have never been satisfactorily confirmed.

Koch likewise tested the immunizing value of tubercle bacilli boiled in dilute mineral acids or strong alkalies; other workers have simply boiled or heated them to 70° C.; others have treated them with glycerine or 25 per cent. lactose; Löwenstein used formalin, Loeffler applied dry heat, Rappin used sodium fluoride, Noguchi sodium oleate, Deycke and Much choline and neurine, but all without result. At a later date Dreyer treated the bacilli with formalin and subsequently extracted them with acetone and as a result the organisms lost their acid-fast property. This so-called *denatyl* vaccine has not been found of value for immunizing guinea-pigs (Bronfenbrenner and Straub 1925, Douglas, J. S. C. *et al.* 1927, Dreyer, Vollum, and Ammitzboll 1931) have reported favourable results from its use in the vaccination of cattle.

The general outcome of most of these attempts has been disappointing, and Calmette, in particular, has concluded that dead vaccines are useless for purposes of immunization. How far this conclusion is justifiable, it is difficult to say. Most of the earlier workers were undoubtedly hoping for the production of an immunity similar to that obtainable in certain other diseases. The knowledge that we have since gained shows that such a hope was unduly optimistic. Recent experiments, made mostly on guinea-pigs with heat-killed bacilli, suggest very strongly that vaccination with dead organisms does lead to a definite increase in the resistance of the animals to infection, as judged by comparison of mortality, average survival time, and certain other criteria, with those in control unvaccinated animals (Petroff 1927, Petroff, Branch, and Jennings 1929, Freund 1932, Hughes 1933, Westenrijk 1933, Branch and Enders 1935, Schwabacher and Wilson 1937, Opie and Freund 1937, Griffith and Glover 1939, Opie, Flahiff and Smith 1939, Smithburn 1939, Wells, Flahiff and Smith 1944), but this increase of resistance falls far short of absolute immunity.

An attempt to improve the antigenic quality of the vaccine has been made by Spahlinger (1932), who grows the bacilli on media containing proteins specific to the type of animal to be vaccinated, and allows them to die out naturally during a period of some months. Adequately controlled experiments with this vaccine have not yet been made, but there is evidence to suggest that it may have some action in raising the resistance of animals to infection (Report 1935*a*). The use of living virulent bacilli for vaccination was tried on a small scale by Webb and Williams (1911) (see also Baldwin and Gardner 1921), but was given up as being too dangerous.

Vaccination with the B.C.G. Strain.—Believing that dead tubercle bacilli are useless for purposes of immunization, and that acquired immunity depends upon the persistence in the body of living bacilli, Calmette introduced a living vaccine consisting of a bovine strain that had become avirulent as the result of several years' subculture on a glycerol bile potato medium. This B.C.G. (Bacille Calmette-Guérin) strain can be injected into animals without giving rise to more than a small retrogressive local lesion, and its presence in the body usually renders the animal sensitive to tuberculin. According to Calmette, calves and heifers vaccinated with 50 mgm. subcutaneously become resistant to tuberculosis, and during the following year are able to withstand an intravenous injection of 5 mgm. of living virulent tubercle bacilli, sufficient to kill unvaccinated animals in 2 months.

The success of his laboratory experiments led Calmette to the vaccination of human subjects. In order to forestall natural infection, he considered it important to vaccinate infants directly after birth. For this purpose he recommended 3 doses, each of 10 mgm., to be given by the mouth on alternate days during the first 10 days of life. Moreover, in order to prevent infection before immunity had become established, he recommended that the infant should be removed from all sources of infection for 2 months after vaccination.

This vaccine has now been tried out on an extensive scale with results that are extremely difficult to assess. The great difficulty is that, with one or two exceptions to be mentioned later, there has been a general failure to realize the necessity of comparing the vaccinated infants with a control group belonging to the same social class, brought up in the same environment, and treated in exactly the same way as the vaccinated group. The usual practice has been to compare the tuberculosis or general mortality in the vaccinated infants with the estimated mortality for non-vaccinated infants in similar surroundings or in the country as a whole. Such comparisons, as Greenwood (1928), Rosenfeld (1928), Wolff (1930*a*, *b*), and Berghaus (1930, 1931) have pointed out, are grossly unfair. In the first place no exact figures exist for the mortality in unvaccinated infants brought up in a tuberculous environment, and the estimates that have been made differ enormously. In the second place, the unvaccinated infants are not strictly comparable with the vaccinated group, partly because they are not kept sheltered from infection during the first 2 months of life, and partly because they receive less parental, medical, and nursing attention than those infants which, after vaccination, are kept under more or less continuous observation. For these reasons, the reproduction, even if it were possible, of the immense pile of data that has accumulated on this subject in France and French-speaking countries, would serve no useful purpose, since from a statistical point of view it is practically worthless.

The realization of these fallacies has now led to the institution by other workers of a few experiments in which an attempt has been made to provide a control group with which the vaccinated group can be more strictly compared. It must be noted that the difficulties of obtaining an absolutely identical control group are almost insuperable, and some allowance has therefore got to be made when considering the results. We have already (p. 1331) drawn attention to the favourable results recorded by Heimbeck following the vaccination of von Pirquet-negative probationer nurses with B.C.G. Similar results have also been obtained by Scheel (1935) with medical students. We may now briefly consider the data collected by other workers on the vaccination of infants.

Park, Kereszturi, and Mishulow (1933) in New York vaccinated infants born into tuberculous families with B.C.G., the vaccine being given by the mouth during the first 10 days of life. A control group of infants was left unvaccinated. The two groups were not strictly comparable, (1) because the vaccinated infants were removed from their parents for 1 month after immunization, while the control infants were left at home; and (2) because the control infants were rather more heavily exposed to tuberculous infection than the vaccinated group. Some of the infants were kept under observation for 5 years, but with most of them the observational period was shorter. Altogether 3 out of 239 vaccinated, and 7 out of 189 control infants died of tuberculosis. The non-tuberculous mortality, on the other hand, was considerably higher in the vaccinated than in the control group—in contradiction of Calmette's assertion that B.C.G. vaccination lowers both the tuberculosis and the general mortality. Another series of children from tuberculous families was vaccinated parenterally—the vaccine being given intracutaneously

or subcutaneously some time after birth. All the vaccinated children reacted negatively to tuberculin before immunization. Of the control children, some had a positive and some had a negative reaction. During the observational period none of the 150 vaccinated children died of tuberculosis. Of the 155 control children with an initially positive Mantoux reaction 5 died of tuberculosis, while of 269 control children with an initially negative reaction 4 died of tuberculosis. While admitting that the differences are not significant, the authors conclude that B.C.G. vaccination apparently confers some measure of protection against tuberculosis in early life. A further report was published by Koreszturi and Park in 1936. The tuberculosis mortality among 690 vaccinated children was 0·9 per cent. against one of 2·7 per cent. among 755 control children. The odds against an observed difference of this magnitude being due to chance alone were estimated at more than 100 to 1.

A similar experiment has been reported by Aronson and Dannenberg (1935), in which both white and coloured infants were observed. No data, however, are provided to indicate how the children were selected, or what degree of similarity existed between the control and the vaccinated groups. The vaccinated infants were given B.C.G. by the mouth during the first 10 days of life. Observations on the infants were made as far as possible at 3-monthly intervals during the first year, and at 6-monthly intervals subsequently. The results are summarized in Table 106.

TABLE 106

DEATHS FROM TUBERCULOSIS IN CHILDREN VACCINATED WITH B.C.G. AND IN A CONTROL GROUP. (Aronson and Dannenberg 1935.)

Type of Exposure.	Vaccinated Group.			Control Group.		
	No.	Mean Age at Time of Last Observation in Months.	Deaths from Tuberculosis.	No.	Mean Age at Time of Last Observation in Months.	Deaths from Tuberculosis.
Exposed to contact with tuberculous patients having a positive sputum	41	31·4	1	84	40	10
Exposed to contact with tuberculous patients having a negative sputum	15	28·3	0	45	39·3	2
Not exposed to known contact with tuberculous patients	14	—	0	38	—	0

The numbers are few, and it will be observed that the mean age at the time of last observation was higher in the control than in the vaccinated group. Since tuberculous mortality is higher in the second than in the first year of life, this may obviously have affected the control group unfavourably. The results, however, do suggest that B.C.G. vaccination tends to confer some protection against the risk of death from tuberculosis during the first year or two of life. Radiographical observations, moreover, showed that whereas only 16·6 per cent. of 36 vaccinated children in the first group had lesions in the lungs, 56·6 per cent. of 83 control children in the same group showed pulmonary lesions.

Neither of these investigations yielded very striking results, but both of them afford some evidence that B.C.G. vaccination may bring about a slight increase in resistance to tuberculosis. How far even this conclusion is justifiable, however, may be questioned. Levine, Vogel and Rosenberg (1938), for example, report the results of a study of 1,830 children of tuberculous families in New York City. Between 1926 and 1932 the children were divided into two equal groups, one of which was vaccinated with B.C.G., the other of which was not. The result of

this was that the children of the more intelligent and co-operative parents tended to be vaccinated and those of the careless and less intelligent parents to constitute the control group. From 1933 onwards, therefore, the children were vaccinated alternately without any selection whatever. The results are shown in Table 107.

TABLE 107

RESULTS OF B.C.G. VACCINATION BEFORE AND AFTER ALTERNATE SELECTION OF CONTROL AND VACCINATED CASES.

(Levine, Vogel and Rosenberg 1938).

Period.	Alternate Selection.	Vaccinated Group.			Control Group.		
		No.	Tuberculosis Deaths.	Tuberculosis Mortality. %	No.	Tuberculosis Deaths	Tuberculosis Mortality. %
1926–32.	No	439	3	0·7	527	16	3·0
1933–36.	Yes	383	6	1·6	361	4	1·1

In the first period the tuberculosis mortality of the controls was over 4 times that of the vaccinated children. In the second period no such difference was apparent. The authors draw attention to the necessity in carrying out comparative trials of this nature of selecting alternate children for vaccination, of studying the degree of exposure of each child to infection, and of carrying out an autopsy on every child that dies to learn the true cause of death. Without such precautions the results are liable to be misleading.

Even if we admitted the beneficial effect of B.C.G. vaccination, which we are not prepared to do, we should still have to decide what value it was likely to be in practice. Tuberculosis mortality has been falling so rapidly, particularly in the lowest age groups, that in this country it is now of very much less importance than it used to be. In 1937, for example, only 1·1 per cent. of the total infantile mortality was due to tuberculosis, and the chances against dying from tuberculosis during the first year of life were about 1,500 to 1. In face of such a slight risk, general vaccination of all infants with B.C.G. is out of the question. If vaccination is to be employed, it will have to be restricted to infants who are born into tuberculous families, and possibly to other classes of the population, such as nurses and medical students, who are exposed to a special risk of infection. The evidence, particularly of Scandinavian and American workers, suggests that vaccination is more effective if performed by the intracutaneous or subcutaneous than by the oral route, and that the degree of allergy resulting is higher. Parenteral vaccination is, however, not without its disadvantages. A cold abscess forms, which persists for months, and may sometimes break down and ulcerate through the skin. How long increased resistance is likely to last it is impossible to say, but Calmette himself advised re-vaccination at 3, 7 and 15 years of age. If re-vaccination is not performed, the immunity—if any—resulting from the primary vaccination will presumably wear off in a year or two. If, on the other hand, it is performed, there is a danger of setting up in a tuberculin-positive patient an acute allergic reaction, which may have serious results.

We may add that there is no evidence to prove that organisms of the B.C.G. strain may regain their virulence by residence in the body. The virulence of the

strain seems to be actually falling, and it is doubtful how long it will retain its protective properties. The Lübeck catastrophe, in which 72 out of 251 vaccinated infants died of tuberculosis (see Report 1935*b*), was almost certainly due to the contamination of the vaccine with another strain of tubercle bacilli. Animal experiments, with the exception of those on cattle (see p. 1347), have on the whole not given a very convincing answer to the prophylactic value of B.C.G. vaccination. Observations on guinea-pigs and mice have shown that B.C.G. vaccination has some beneficial effect, mainly in retarding the development of tubercle bacilli in the body and thus favouring the occurrence of a more chronic type of disease (Okell and Parish 1928, Schwabacher and Wilson 1937, 1938, Griffith and Glover 1939, Liebow, Burn and Soper 1940).

A few of the papers in the enormous bibliography that has now arisen on this subject may be quoted (see Calmette 1924, 1930, 1931, 1932, Calmette and Guérin 1924, Calmette *et al.* 1924, 1926, Weil-Hallé and Turpin 1925, Wilbert 1925, Gamna and Giordano 1926, Heymans 1926, Tsekhnovitzer 1926, Gerlach 1927, Kraus 1927, Petroff *et al.* 1927, 1929, Gerlach and Kraus 1929, Kraus and Gerlach 1929, Debré and Cofino 1929, Gerlach 1930, Begbie 1930, 1931, Neufeld 1930, Uhlenhuth and Seiffert 1930, Griffith 1931, Birkhaug 1933, Clawson 1933, Meiszner and Prausnitz 1934, Irvine 1934, Shaffer 1935, Naeslund 1935, Besta and Lemneberg 1935, Rist 1936, Kayne 1936, Holmgren 1936, Jonesco-Mihaiesti *et al.* 1938, Kostic-Jocksic 1939, Baudouin 1940, Cantonnet 1942, Etcheverry 1942).

Vaccination with the Vole bacillus.—It is too early to decide whether vaccination with bacilli of the murine type isolated by A. Q. Wells (1937) from tuberculous voles will play a part in vaccination of human beings against tuberculosis. The preliminary observations of Wells and Brooke (1940) on guinea-pigs and of Griffith and Dalling (1940) on guinea-pigs and calves suggest that it is capable of giving rise to a greater degree of immunity than the B.C.G. strain. The results of further tests will be eagerly awaited.

Therapeutic Immunization against Tuberculosis.—The introduction of tuberculin by Koch in 1890 was the first of a long series of attempts to cure tuberculosis by the use of vaccines of tubercle bacilli or their products. Koch's old tuberculin, which has already been described (p. 1327), gave rise to such severe reactions—both focal and constitutional—that it was for a time practically abandoned. It is now used chiefly for stimulating chronic cases of tuberculosis that have passed into a torpid state, in which neither advance of the disease nor progress towards recovery is occurring (Burnand *et al.* 1922). Extremely small doses are employed. Jaquerod (see Burnand *et al.* 1922) starts with 0·1 ml. of a 1/100,000 dilution of old tuberculin; he repeats the injection every 2 days, increasing the dose by 1/10 ml., provided the reaction is not too severe. After about 5 months the patient is able to withstand 0·5 ml. of the undiluted tuberculin; this dose is repeated 3 or 4 times, after which the treatment is finished. The fundamental rule of all tuberculin treatment is never to provoke a febrile reaction of any importance.

Starting from the observation that patients suffering from lupus seldom contract serious lung disease, von Aichbergen (1937) recommends the artificial production of lupus for the treatment of severe pulmonary tuberculosis. Virulent tubercle bacilli are rubbed on the scarified skin of the patient, and the inoculations are repeated as often as necessary. Lupoid or verrucous lesions appear and remain localized. No focal reactions have been observed in the lungs. Favourable results are claimed for this method of treatment.

Chemotherapy of Tuberculosis.

Rich and Follis (1938) and Buttle and Parish (1938) reported the results of experiments on guinea-pigs suggesting that sulphanilamide had a mildly favourable effect on the course of the disease; but Smithburn (1938) and Steinbach and Dillon (1939) working with sulphanilamide, and Dietrich (1938) working with prontosil, found no beneficial effect at all.

More recently Promin (sodium *p. p'*-diaminodiphenylsulphone-N, N'-diglucose sulphonate) has come under trial. Observations on guinea-pigs recorded by Hinshaw and Feldman (1941) and Feldman, Mann and Hinshaw (1942) seem to show that it is of therapeutic value, but the results so far obtained by its use in man have not been promising (Zucker, Pinner and Hyman 1942).

TUBERCULOSIS IN ANIMALS

Frequency and Bacteriology.—Tuberculosis is widespread throughout the animal kingdom. It is common among certain species of domesticated animals, and though rarely encountered in wild animals living under feral conditions, it is not infrequent among them when they are brought into captivity (see Griffith 1928*a*, *b*, 1939, Scott 1928, Scott and Beattie 1928). Cattle, pigs, and fowls are the most frequently affected of stock animals. According to Myers (1940) tuberculosis in fowls is more common than is generally supposed. Dogs and cats suffer not infrequently (see Hjarre and Herlitz 1936, Hjärre 1939), horses occasionally, and sheep apparently seldom.

Bacteriologically, human, bovine, and avian types are found with varying degrees of frequency (see Table 108 which has been compiled from the results of A. S. Griffith 1924, 1925*a*, 1926, 1928*a*, *b*, Stableforth 1929, Cornell and A. S. Griffith, 1930, Lovell 1930, Lovell and White 1940, 1941, Schwabacher 1934, and Hjärre 1939, together with some of our own results).

Cattle are almost invariably infected with the bovine type, usually from other animals, but occasionally from a tuberculous attendant (Bang and Jepsen 1936, Nielsen and Plum 1940, Hedvall 1942). The avian bacillus may give rise to localized lesions, particularly in calves (see Minett 1932, Gloyne 1933, Glover and Griffith 1934–35); it has been isolated from the sputum, milk, intestine, and mesenteric glands (Wolters 1931, Glover and Griffith 1934–35, Glover 1941). Pigs suffer from generalized and from localized infection with the bovine, and from localized infection with the avian and much less often the human type. Nearly all strains isolated from the horse have been of bovine type; but they are often atypical and of less than standard virulence for calves and rabbits (Griffith 1937*b*). In the dog the human type seems to be commoner than the bovine (see Lovell and White 1940); but all strains from the cat have proved so far to be of bovine type. Sheep may be infected with the bovine or avian type; in the United States Harshfield, Roderick and Hawn (1937) found the avian type in 25 out of 26 cases. Parrots kept as pets are usually infected with the human type, but those living in aviaries with other birds may be infected with the avian type (see Cobbett 1917). Fowls and most other birds are infected almost exclusively with the avian type of bacillus.

Tuberculosis of Cattle.—Tuberculosis is one of the most serious diseases of cattle. In this country it is extremely common. As already mentioned, about 40 per cent. of cattle react to the tuberculin test, about 40 per cent. of animals slaughtered in

TABLE 108

TYPES OF TUBERCLE BACILLI FOUND IN CERTAIN DOMESTICATED AND CAPTIVE WILD ANIMALS
(Mainly English Investigations.)

Species of Animal.	No. of Cases.	Types of Tubercle Bacilli.		
		Bovine.	Human.	Avian.
Anthropoid Apes	6	—	6 [1]	—
Lemurs	2	—	2	—
Monkeys	26	8	18 [2]	—
Cattle	52	50	1	1
Horse	43	42 [3]	—	1
Sheep	17	14	1	2
Goat	13	13	—	—
Deer	3	3	—	—
Gnu	3	2	1	—
Antelope	3	3	—	—
Pig	258	183 [4]	5 [5]	70 [6]
Cat	34	33 [7]	1	—
Dog	116	34	82	—
Rabbit	8	4	—	4
Guinea-pig	6	5	1	—
Ferret	1	1	—	—
Parrot	1	—	1	—
Fowls, and other birds	20	—	1	19

[1] Including 1 strain of sub-standard virulence.
[2] Including 1 dysgonic strain.
[3] Including 17 strains of sub-standard virulence.
[4] Including 4 cases in which avian, and 1 case in which human, bacilli were also present.
[5] Including 1 case in which bovine bacilli were also present.
[6] Including 4 cases in which bovine bacilli were also present.
[7] Including 1 strain of sub-standard virulence.

public abattoirs show macroscopic lesions of tuberculosis, and about 0·5 per cent. of all milch cows excrete tubercle bacilli in their milk (Report 1932*a*, 1934, Savage 1933). The disease is common throughout Europe, but in North America it is very much less common. In the United States the proportion of tuberculin-positive animals has now been reduced to less than 0·5 per cent. as the result of a slaughter policy instituted by the Federal Government in 1917 (Myers 1940). In the tropics the disease is more frequent than has been generally supposed (Carmichael 1939).

The incidence of the disease increases with the age of the animal; it is uncommon in calves (see Gofton 1937), fairly common in bullocks and heifers, and very common in cows—particularly the older animals. Table 109, compiled by Delépine (see Cobbett 1917) on the basis of tuberculin tests controlled by post-mortem examination, will make this clear.

The organs most frequently infected in cattle are the lungs, pleura, and thoracic lymph glands; less often the lymph glands and organs of the abdominal cavity. Tuberculosis of the udder is frequent, especially in the later stages of the infection. According to Vallée (1905), who gives records of over 43,000 tuberculous cattle, pulmonary lesions were present in 75 per cent., and hepatic lesions in 28 per cent., of all cases of localized tuberculosis; in generalized tuberculosis the lungs were always invaded, and the liver was invaded in 83 per cent. of cases. It is important to realize that tuberculous cattle may excrete bacilli in their milk even though no

TABLE 109

FREQUENCY OF TUBERCULOSIS IN CATTLE CLASSIFIED ACCORDING TO AGE.

Age.	Number Examined.	Percentage Tuberculous.
0–1 years	29	3·4
1–2 „	68	13·2
2–3 „	112	24·1
3–5 „	51	23·5
5–9 „	94	48·9
9–13 „	25	76·0

evidence of disease of the udder, as judged by microscopical examination after death, is to be found (Report 1909, Gaiger and Davies 1933). Cattle form the chief source of infection for other domesticated animals.

Diagnosis of Tuberculosis in Cattle.—The diagnosis of tuberculosis in cattle follows much the same lines as that of human tuberculosis. An attempt may be made either to demonstrate tubercle bacilli directly in the animal's secretions or excretions, or to arrive at an indirect diagnosis by some form of immunity reaction, such as the tuberculin test, which, if positive, may be interpreted as evidence of infection. The direct method is restricted largely to the examination of sputum and milk. Sputum is not always easy to obtain, but if the cow has a cough, small particles of sputum can often be found on the wall of the byre opposite the animal's head (Riddoch 1903).

Demonstration of Tubercle Bacilli in Milk.—Microscopical, cultural, serological, and animal inoculation tests are available, the last method being the commonest and most delicate.

Microscopical examination is of value mainly for the milk of individual cows suspected of suffering from udder tuberculosis.

The most satisfactory method is to draw off quarter samples from the udder, and to spin each sample separately a short time at low speed, to spread a film on a slide, to fix and de-fat with a combination of heat, alcohol and ether, and to stain with carbol-fuchsin (see Cowan and Maddocks 1935). The work of Torrance (1922, 1927), Matthews (1931), and Davies (1933) has shown that tubercle bacilli in milk are generally associated with the presence of characteristic endothelial cell clumps. It is therefore only necessary to examine the film with a low power for the presence of these clumps, and to reserve the oil-immersion lens for films in which these clumps are present. The clumps themselves are not diagnostic of tuberculosis, but they afford a good indication of the locality of the tubercle bacilli in the film. It would appear that about 50 per cent. or so of milks from individual cows shown to contain tubercle bacilli by guinea-pig inoculation can be detected by the microscopic method (Cowan and Maddocks 1935).

Cultivation is not often used as a routine, since it is less delicate than the guinea-pig method and not as rapid as the microscopic method. It is suitable mainly for the milk of individual cows collected under aseptic conditions.

The sediment resulting from high-speed centrifugation should be treated with an equal volume of 15 per cent. HCl for 10–15 minutes, and inoculated on to suitable media (see Wolters and Dehmel 1931).

The main value of the method seems to be in the occasional detection of the avian tubercle bacillus, which might be missed by the animal inoculation method.

Serological methods are still in the experimental stage. German workers have reported favourably on the value of the complement-fixation test carried out on the milk whey in the diagnosis of udder tuberculosis (see Karsten 1933, 1935, Sontgen and Menck 1933, Rautmann 1934, Rautmann and Hartwigk 1934, Schultz 1937, Paarmann 1936, Kuhlmann 1936), but there is little evidence to suggest that they furnish a reliable index of the infection of the milk.

Guinea-pig inoculation constitutes the most generally useful and most delicate method of detecting tubercle bacilli in milk.

About 50–100 ml. of milk, either mixed or from individual animals, should be centrifuged at 3,000 r.p.m. for 30 minutes, after removal of the gravity cream. The sediment and the cream should be mixed, and 3 ml. of the mixture should be injected intramuscularly into the left thigh of each of two animals, which should previously have been shown to be free from tuberculosis by means of the intradermal tuberculin test. One animal should be killed 3–4 weeks after inoculation; if the results are negative, the other animal should be left for 6–8 weeks. The post-mortem appearances of animals inoculated with tubercle bacilli have already been referred to (p. 1325), and it need only be mentioned here that no animal should be regarded as tuberculous unless characteristic lesions containing acid-fast bacilli are demonstrated.

The Tuberculin Test in the Diagnosis of Cattle Tuberculosis.—This test may be carried out in a number of different ways, but each method consists in noting a reaction, local or constitutional, to the injection of tuberculin. In this country the double intradermal test is generally used, but in the United States the single intradermal test, made by inoculating tuberculin into the skin of the caudal fold, is officially recommended. For the full technique of the double intradermal test the following references may be consulted (Report 1925, Buxton and MacNalty 1928).

Briefly the test consists in injecting 0·1 ml. of old tuberculin into the deeper layers of the dermis of a shaved area on the side of the neck. The animal is examined 48 hours later, and if a large diffuse œdematous swelling is found, hot and tender, the reaction is regarded as positive. If no more than a pea-like swelling is present, a second injection of 0·1 ml. should be made into the centre of the nodule, and the animal should be inspected after a further 24 hours. The true criteria of a positive reaction are swelling, heat, and tenderness, and as these are liable to variation, the interpretation of the test demands considerable experience.

A positive tuberculin reaction generally denotes that the animal contains a tuberculous focus somewhere in the body. It gives no information on the activity of the focus. The test is used mainly to determine whether the animal is *infected*, not whether it is *infective*. A negative reaction, while not absolutely excluding the presence of infection, is strong evidence against it. The test has been severely criticized by a number of workers, and there is no question that it is not entirely satisfactory. The presence of infection with avian tubercle bacilli, which appears to be not uncommon, is sufficient to give rise to a positive reaction, even though avian bacilli rarely cause more than a localized, retrogressive type of tuberculosis. Similarly, a reaction may sometimes be obtained in animals showing skin infiltrations and nodules due to an acid-fast bacillus different from the tubercle bacillus; this condition has been described in the United States (see Daines and Austin 1934) and in Great Britain by Robertson and Hole (1937). The test, it will be understood, is not absolutely specific for tuberculosis, and is often given by animals containing insignificant foci of disease. Border-line reactions are not infrequent; possibly the replacement of old tuberculin with a purer type of tuberculin may

diminish the difficulty caused by non-specific reactions (Montgomerie and Thomson 1939). In careful hands, however, it is of very real value, and is indispensable in the detection of tuberculous infection (see Teipel 1925, Ernest and Lash 1928, Haring and Traum 1929, Hastings *et al.* 1930, 1933, Mohler 1931, Fowler and Wright 1931, Glover 1932–33, Buxton and Glover 1932–33, Minett 1932, McEwen and Roberts 1934, Cabot 1938, Montgomerie and Thomson 1938, Parish 1938, Buxton and Glover 1939).

Control of Tuberculosis in Cattle.—Opinions on the measures to be taken to control tuberculosis in cattle are very divided. In this country (see also p. 2050) and in Germany the practice has been to rely mainly on the detection of tuberculous animals by clinical inspection, combined with bacteriological examination of the milk, and to remove all open cases of disease. Though this method may, if prosecuted with real thoroughness, bring about a diminution in the total number of animals suffering from open tuberculosis, it probably has little or no effect on the total incidence of infection. The reason for this, of course, is that by the time a tuberculous animal has been diagnosed by the methods just described, it has already been excreting tubercle bacilli for some time and has acted as a focus of infection for other animals in the herd. In Germany and Switzerland the similar but more thorough method of von Ostertag (1913, 1936) has been used; this makes use of bacteriological examination for the detection of animals showing no clinical evidence of tuberculosis. The method claims not to eradicate the disease, but to pave the way for the slaughter policy based on the tuberculin test (see Brook 1937).

In the Scandinavian countries, largely owing to the initiative of Bang (see Bang 1928, 1929, 1930, 1932), and in the United States and Canada, a more radical method has been adopted, aiming at the elimination not only of open cases of tuberculosis, but of all animals infected with the tubercle bacillus. The general method is to test every animal in the herd with tuberculin, to segregate, remove, or slaughter the positive reactors, and to build up the herd exclusively from negatively reacting animals. The tuberculin test must be repeated at 3- or 6-monthly intervals to detect any fresh reactors in the clean herd. This method, if accompanied by complete removal or slaughter of the reacting animals, is often highly successful in eradicating tuberculosis and in maintaining a tuberculosis-free herd. In the United States, for example, the proportion of animals reacting to tuberculin was reduced between 1917 and 1940 from an average initial rate of about 4 per cent. to one of about 0·5 per cent. (Myers 1940). Where segregation alone is practised, and two herds are kept on the same farm (Bang's method), the results are less satisfactory.

The respective merits of the different methods of controlling tuberculosis in cattle cannot be fully discussed here; opinion is sharply divided upon them. The eradication method is undoubtedly much the more effective, and should be recommended whenever sufficient money is available to meet the cost. The cost, however, is a very real consideration, and many agriculturists regard tuberculosis as of insufficient economic importance to justify a radical scheme. They would rather rely on the rapid detection of open cases of tuberculosis, and the restriction of the herd to comparatively young animals, in which the disease is less frequent than in the older animals (see Table 109, p. 1344). The question must be decided by the agriculturists themselves. From the point of view of human medicine, safeguarding of the milk supply is much more satisfactorily effected by pasteurization than by

reliance on any eradication scheme (see Haring 1929, Mohler *et al.* 1929, Hutyra 1929, Günzel 1929, Glover and Spencer 1930, Seiferle 1930, Regler 1930, Heath 1930, Wight 1930, Kiernan and Wight 1930, Jordan 1933).

Vaccination of Cattle against Tuberculosis. If an eradication scheme is impracticable, it is clearly desirable to aim at the diminution of advanced and open cases of tuberculosis. Besides the measures already discussed for this purpose, the possibility of vaccinating the animals so as to raise their general level of resistance to tuberculous infection has long occupied the minds of veterinary and other workers. Von Behring introduced the method of vaccinating calves with living bacilli of the human type. The method was successful in rendering the animals allergic, and fairly successful in protecting them—for a few months at least—against natural tuberculosis; but it had to be discarded, since it was found that the bacilli remained alive in the tissues and were excreted by the milk, which was, of course, dangerous for human beings (Report 1911*c*, Griffith, A. S., 1912–13, 1916–17). Attempts have been made to vaccinate cattle with avian bacilli, but the results have not been so successful—probably because avian bacilli do not set up the same focal lesions in cattle that human bacilli do (M'Fadyean *et al.* 1913, Gamna and Giordano 1926). Friedmann introduced his turtle bacillus for vaccination, but this organism was found to provide little or no protection for warm-blooded animals.

More recently the claims put forward by Calmette for his B.C.G. vaccine have attracted considerable attention. Many different workers have now carried out experiments to test the prophylactic value of this vaccine. Since the technique of different workers has varied very considerably, it is impossible to summarize quantitatively the results that have been obtained, and we must refer our readers to the original papers in which they are described (see Watson 1928, 1930, Schroeder and Crawford 1928–29, Rankin 1929, Rankin *et al.* 1932, Haring *et al.* 1930, Bang, O. *et al.* 1931, Cotton and Crawford 1932, Jundell and Magnusson 1933, Buxton and Griffith 1931, Griffith, Buxton, and Glover 1931, 1935, Buxton, Glover and Griffith 1934–35, Ascoli 1936, Buxton 1936, Buxton *et al.* 1939).

The results, however, are sufficiently concordant to justify a few tentative general conclusions. There seems to be little doubt that if B.C.G. is given to uninfected calves shortly after birth, preferably by the parenteral route, their resistance both to experimental and natural infection with the tubercle bacillus is definitely increased compared with that of unvaccinated control animals. The immunity produced is by no means absolute, and does not seem to be sufficient as a rule to prevent actual infection from occurring. It is, however, often sufficient for a time to keep the infection from becoming active. There is evidence that the immunity resulting from the primary vaccination gradually diminishes with passage of time, and wears off to a greater or less extent after 12 months. Re-vaccination may partially restore the lost immunity, but there is not yet sufficient evidence to justify an opinion on its real value.

From these results it is clear that vaccination of calves with B.C.G. deserves a trial under field conditions. Whether the limited increase in immunity that it is likely to produce will be sufficient to bring about any considerable diminution in the number of active cases of tuberculosis among animals that are exposed to frequent infection from their fellows, it is impossible to predict, and we must await the verdict of actual experience. It is important to realize that B.C.G. vaccination renders the animal allergic, so that the tuberculin test can no longer be used to indicate the presence of infection with virulent bacilli. B.C.G. vaccination is

therefore incompatible with an eradication policy. The promising results obtained by Griffith and Dalling (1940) in the vaccination of calves with the vole strain of tubercle bacillus have already been referred to (p. 1341).

Mention may perhaps be made of the Spahlinger vaccine (see p. 1337) which has been credited with some success in preliminary experiments in the North of Ireland (Report 1935*a*).

Tuberculosis of Pigs.—The exact incidence of this disease is unknown, but slaughter-house records leave no doubt of its frequency in this country. Ellison (1930) found that 12·09 per cent. of 72,350 pigs supplied to a bacon factory at Ipswich showed macroscopic lesions of tuberculosis. Other figures showed that 7·6 per cent. of 44,000 pigs coming into another bacon factory over a period of 2 years contained lesions of tuberculosis (Report 1933*b*). The glands draining the alimentary tract are mainly affected, particularly the submaxillary and mesenteric group. In about 84 per cent. of carcasses in Ellison's series the lesions were confined to the head and neck, and in about 5 per cent. to the plucks or offal or both. In only 0·15 per cent. of animals was the disease generalized or advanced.

The bovine type of tubercle bacillus is found in about two-thirds and the avian bacillus in about one-third of the cases ; the human tubercle bacillus is very much less common (Griffith, A. S., 1928*a*, Cornell and Griffith 1930). Submaxillary glands infected with avian or human bacilli are often not appreciably enlarged, and contain discrete caseo-calcareous tubercles ; those infected with bovine bacilli are generally enlarged and composed wholly or in part of caseous or caseo-calcareous material surrounded by fibrous tissue. Generalized tuberculosis is almost invariably due to the bovine type.

Pigs become infected from various sources, the chief being raw cow's milk, infected cow dung, slaughter-house offal, and the droppings of tuberculous fowls. Infection occurs by the mouth. The disease can be diagnosed during life by the intradermal inoculation of tuberculin into the ear, but in most animals infection is not diagnosed, and often not even suspected, till they come to the abattoir. Prevention consists in avoiding contamination of the food with tubercle bacilli ; boiling all food, including milk, that may possibly be infected ; and keeping the animals separate from tuberculous cattle and poultry (see Mohler and Washburn 1930). In the United States an active campaign based on the tuberculin test is now being waged against the disease (Myers 1940).

Tuberculosis in Fowls.—Comparatively little is known of the frequency with which tuberculosis attacks fowls in this country. It is met with in farmyard birds, but is uncommon on poultry farms. In Germany, however, Klimmer (quoted by Knorr 1932) stated that of 75 million hens, 7½ million were tuberculous, and that 3 per cent. of market eggs, and 10 per cent. of eggs from tuberculous hens, contained tubercle bacilli. In the United States a survey of 115,700 flocks of poultry in 40 States revealed the presence of tuberculosis in 6,690 (5·8 per cent.) ; the actual number of fowls affected was estimated at over 8 million (Mohler and Washburn 1930). The highest incidence of infection occurs in the north central states. Disease is much commoner in old birds than in young (Feldman 1938). The organs most frequently affected are the liver, spleen, intestines, lungs, bones, joints, peritoneum, kidneys, and ovary (Gallagher 1926). The disease can be diagnosed by the intradermal tuberculin test using avian tuberculin. The slaughter policy seems to be the most suitable method of dealing with the disease when it is extensive, but an eradication plan based on the use of tuberculin may be tried

if the disease is less extensive or the birds valuable. (For a general survey of avian tuberculosis see Gloyne 1933, Feldman 1938.) Acid-fast organisms have been reported in bumble-foot of chickens, but their exact nature is unknown (see Bunyea 1936).

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CHAPTER 60

LEPROSY, RAT LEPROSY, AND JOHNE'S DISEASE

LEPROSY

History and Epidemiology.—Leprosy is a disease of great antiquity; when or where it originated is not known, but according to Rogers and Muir (1925) it is probable that the original focus was in the northern belt of Central Africa. The disease was described in Asia Minor about 345 B.C.; thence it spread gradually westwards, till in the Middle Ages practically every country in Europe was affected. During the fourteenth and fifteenth centuries leprosy in Europe declined rapidly; about the same time, however, it was carried to the Western Hemisphere. During the last 70 years the disease has appeared in Oceania, where it has spread widely; in Hawaii, for example, it was estimated in 1909 that 1 in every 40 natives was a leper (Brinckerhoff and Moore 1909).

At the present time the highest incidence of the disease is confined to tropical countries, particularly (1) Equatorial Africa; (2) the long belt stretching from Assam and Burma, through the Malay States and Indo-China to the islands of the Pacific; and (3) the West Indian Islands and the northern part of South America. Males appear to be more commonly affected than females. Infection seems to be most frequent in youth and early adult life; Rogers and Muir (1925) state that probably over 50 per cent. of cases are infected before the age of 20. The lower social classes are more frequently infected than the higher. The incubation period may be almost any time, from a few weeks to several years; the commonest period is 2 to 4 years, but it is said that it may extend even to 40 years. The disease is extremely chronic and may be of lifelong duration; after it has lasted for a variable length of time it may retrogress as the result of a naturally acquired immunity; on the other hand, after remaining chronic for years, it may take on an acute course. The mortality from the disease itself is comparatively low.

The way in which leprosy is conveyed is uncertain. Most workers are agreed that it is infectious, but the manner in which infection is spread is still largely a matter of conjecture. Considering the long incubation period of the disease, this is not surprising. The restriction of the lesions to the skin suggests that the bacilli gain entrance through cuts, abrasions, and fissures, but it is possible that the nasal mucosa may be primarily infected. Lepers suffering from the tubercular form are very much more infective than those suffering from the anæsthetic form; this is because lepra bacilli are numerous in the nodules of the skin, but are uncommon in the anæsthetic patches. Blood-sucking flies and other insects have been suspected as carriers of infection, but no convincing evidence against them has been brought forward. On the whole the infectivity of the disease

is low; close and long-standing contact with lepers has generally to be maintained before infection is contracted.

The **bacteriology** of the disease has already been described in Chapter 16.

Experimental Reproduction of the Disease in Animals.—A few workers claim to have reproduced leprosy in monkeys.

Nicolle in 1905 inoculated two bonnet monkeys subcutaneously in front of the left ear with ground-up tissue from a patient with generalized nodular leprosy. A nodule appeared 62 days later at the site of inoculation, and a fortnight after its appearance it was partly excised; histologically it consisted of several small nodules made up of lymphocytes and mononuclears; there were no giant cells and no caseation; lepra bacilli were present in moderate numbers, almost exclusively within the cells. Inoculation by scarification of the skin, or by rubbing on to the nasal and conjunctival mucosa, was without effect. More recently, Reenstierna (1926) inoculated two monkeys (*Macacus sinicus* and *M. rhesus*) with a suspension of ground-up lepromata from two untreated patients with generalized nodular leprosy; the inoculations were made in the supraorbital and malar regions of both sides of the face, into both nostrils, and in the pubic region. On the 39th day, a small, round, hard nodule was visible over the right malar bone; similar swellings appeared later over the other sites of inoculation, with the exception of the nasal mucosa, which remained intact. The nodules increased till the 70th day, when they varied in size from a small pea to a nut. Pieces were excised from some of these and injected into guinea-pigs and monkeys; both sets of animals remained well. Microscopically, the nodules consisted of granulation tissue with large mononuclear cells, often vacuolated, containing leprosy bacilli. Shortly after excision of the nodules, the remaining lesions began to decrease in size; ultimately they disappeared. Similar results were obtained with 6 other monkeys. The nodules appeared in 5 to 8 weeks, sometimes reached the size of a small plum, and disappeared as a rule in 6 to 10 weeks. Passage experiments were unsuccessful. Soule and McKinley (1932) likewise found that *Macacus rhesus* and *Cebus olivaceus* monkeys, inoculated intradermally over the eyebrow, developed firm, hard, nodular swellings in 18–20 days, which increased gradually in size for 10–14 days, then retrogressed, and disappeared entirely within another 3 or 4 weeks. The injection of pure cultures, made according to their technique, also resulted quite frequently in the production of transient nodular lesions (see also McKinley 1934).

There appears to be little doubt from these experiments that a localized form of leprosy can be conveyed to monkeys by inoculation of leprous material; but the disease is transient and rapidly cures itself.

More recent observations suggest that some cases at least of human leprosy are caused by the *rat leprosy bacillus*. In 1922 Marchoux (1922, 1933) reported the production of a disease in rats, indistinguishable from rat leprosy, following the injection of a splenic suspension containing abundant acid-fast bacilli from a young Haitian who died from a disease diagnosed as leprosy. Little attention was paid to this finding until Adler (1937) in Palestine, following up the observation of Balfour-Jones (1937) (see also Laidlaw 1937) that rat leprosy could be conveyed to the Syrian hamster (*Cricetus auratus*), described the successful transmission of infection to three of these animals by the inoculation of material from leprosy in man. The following year Burnet (1938*a*, *b*) recorded the production of leprosy in 1 out of 6 hamsters inoculated with human material; and in 1939 Burnet and Jadfard reported one further successful inoculation. Dubois and Gavrilov (1943) inoculated 16 hamsters with material from 12 cases of leprosy; one of these animals developed rat leprosy. The interpretation of these findings is still not clear. Until further work has been carried out, and until the frequency of

natural infection of the Syrian hamster with the rat leprosy bacillus has been ascertained, it would be rash to do more than suggest that this organism may be responsible for occasional cases in man.

Diagnosis.—In the tubercular form, a piece of skin over one of the nodules should be examined microscopically for leprosy bacilli (Fig. 280). With a sharp pair of scissors curved on the flat, a piece of skin 3 mm. long and 2 mm. deep is clipped off the surface. The corium is pressed downwards on to a clean new slide, and a film preparation made, and stained with Ziehl-Neelsen (Rogers and Muir 1925); or the skin may be fixed, embedded in paraffin, and sections cut in the usual way. If acid-fast bacilli cannot be found in the nodules, it is advisable to examine the nasal mucosa. The most satisfactory method is to scrape the mucosa gently with a blunt scalpel, and make ordinary film preparations. The bacilli can frequently be demonstrated in this situation in the tubercular form, and may be found in some cases of the anæsthetic form of the disease. Sometimes they are present in the nasal mucosa before skin nodules have developed; thus they are said to be demonstrable occasionally in apparently healthy contacts. In early cases of generalized leprosy, the bacilli can often be demonstrated in clippings from the lobule of the ear. If there is any doubt as to whether the organisms found in the nose or skin are true leprosy bacilli, it is advisable to inject a suspension of the ground-up material into guinea-pigs; if they are leprosy bacilli, there will be no result; if on the other hand they are tubercle bacilli, the animals will develop the usual form of experimental tuberculosis.

When the lungs are affected, the sputum may contain lepra bacilli, which are most easily distinguished from tubercle bacilli by animal inoculation.

The complement-fixation test has been employed for the diagnosis of leprosy, but it does not appear to be of much value in practice, since a positive reaction may be obtained with antigens made from tubercle bacilli (Row 1925–26).

Lepers frequently give a positive Wassermann reaction; doubtless this is often due to the co-existence of syphilis, but there appears to be little doubt that the Wassermann reaction may be positive in leprosy in the complete absence of syphilitic infection. In countries in which the positive Wassermann reaction rate varies from 7 to 20 per cent. amongst the general population, many observers have found that the rate amongst lepers varies from 15 to 95 per cent. (Rogers and Muir 1925). The great variation in the percentage of positive reactions amongst the leprous population seems to depend largely on the type of antigen employed. Thus Hasseltine (1924) examined the serum of 236 lepers in Hawaii. Using an acetone-insoluble antigen he obtained 17·8 per cent. of positive results; with an antigen consisting of an alcoholic extract of beef heart to which 0·4 per cent. of cholesterol had been added, he obtained 50 per cent. of positives; and with Kolmer's (1922) new antigen he obtained 21·6 per cent. of positives. The greatest number of positive reactions occurred in lepers with the nodular type of

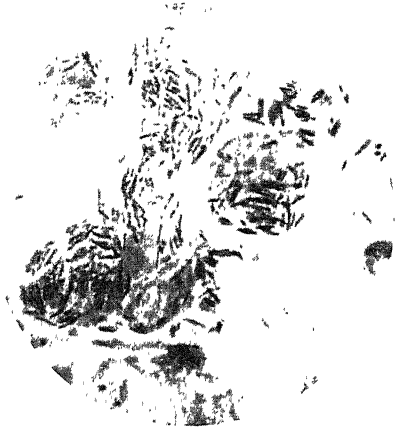


FIG. 280.

Leprosy bacilli in the tissues. The bacilli are seen in dense clumps, mostly intracellular (× 1000).

disease, the smallest number in those with the anæsthetic type. In each form, lepers in the advanced stage of the disease gave more positive results than those in an early stage. Of the general population about 12 per cent. showed evidence of syphilis. It seems clear from these findings that (i) a large number of undoubted lepers give a negative Wassermann reaction, even when a highly sensitive cholesterolated antigen is used; (ii) if a cholesterolated antigen is used, a higher proportion of lepers react positively than when an acetone-insoluble antigen without addition of cholesterol is used. Hasseltine found that many lepers with a positive reaction improved under anti-syphilitic treatment. In the differential diagnosis of leprosy and syphilis, he regards a positive reaction with an acetone-insoluble antigen or with Kolmer's antigen as strong presumptive evidence of syphilis; in the absence of definite evidence of leprosy, such patients should be given anti-syphilitic treatment before being declared lepers. Fletcher (1915), working in the Federated Malay States, obtained 13·6 per cent. of positive Wassermann reactions in lepers with no history of syphilis, and only 3·4 per cent. in non-lepers who also had no history of syphilis. The luetin skin test was uniformly negative in leprosy, even when the Wassermann reaction was positive. Since the luetin test may be negative when the Wassermann reaction is positive, and more positive Wassermann reactions occur in lepers than in non-lepers, Fletcher concludes that leprosy itself may be responsible for a positive Wassermann reaction.

Attempts to diagnose leprosy by means of a specific allergic reaction with a substance known as *lepromin* (Bargehr 1926), or with other substances derived from acid-fast bacilli, have not proved successful (McKinley 1938).

Prophylaxis and Treatment.—The prophylaxis of leprosy is at present a hygienic problem, and rests on the detection and isolation of infected persons, and on their adequate control.

In the treatment of leprosy, vaccines of several different types have been used. Tuberculin was tried as early as 1891, but without result. Nastin is a preparation made by ethereal extraction of an acid-fast streptothrix, and used largely by Deycke; the results do not appear to be very promising. Vaccines have been prepared from the numerous acid-fast bacilli isolated from leprous lesions, also without any marked benefit. With all these vaccines a certain number of patients improve temporarily, but this improvement seems to be largely due to a non-specific reaction, and is most marked when a severe constitutional reaction is produced. Several workers have used the bacilli present in lepra nodules. Hasson (1925–26), for example, employs a vaccine consisting of an autolysate of lepra bacilli, obtained from artificial blisters in lepers, mixed with *Ps. pyocyanea*; this latter organism is added to provoke a febrile reaction. His own reports were very favourable; improvement was said to begin after the third injection, and to be progressive. Patients treated for 18 months had not relapsed. It is doubtful how far this treatment is specific; Row (1925–26) states that he has observed great improvement by the use of a vaccine consisting of autolysed tubercle bacilli.

RAT LEPROSY

Rat leprosy is a disease that was first described by Stefansky (1903) in 1901 at Odessa. Rats were being slaughtered in large numbers, consequent on the outbreak of human plague, and rat leprosy was found in 4–5 per cent. of them. The incidence reported by other observers varies considerably.

In the United States McCoy (1913) found it in 186 out of about 200,000 rats caught in San Francisco, Cal., an incidence of 0·093 per cent.; and Wherry (1908) found it in

20 out of 9,631 rats caught in Oakland, Cal., an incidence of 0·21 per cent. In Japan, Ota and Asami (1932) found it in 0·7 per cent. of *Rattus norvegicus* and in 0·1 per cent. of *Rattus rattus*; and Yamamoto, Sato and Sato (1936) found it in 1·24 per cent. of 2,573 rats caught in Tokyo. According to Marchoux (1933), about 5 per cent. of the sewer rats in Paris harbour rat leprosy bacilli in the lymph nodes, but in only about 0·6 per cent. of the rats are generalized lesions present. In Batavia 9·3 per cent. of about 10,000 rats were said by Lampe and de Moor (1936) to have lesions of the lymph nodes, but not more than 0·23 per cent. of the animals had manifest skin leprosy.

The disease exists in two forms—the glandular type and the musculo-cutaneous type—but there appears to be no sharp division between them. In the *glandular type* one or more of the groups of subcutaneous glands—inguinal, axillary, or cervical—is enlarged, hard, and of whitish colour. On section the glands are uniform and hard; there are no nodules or necrotic areas visible macroscopically. Microscopically, the capsule and trabeculæ are thickened; the sinuses are filled with dense aggregations of irregularly polygonal cells, which have a large nucleus and much cytoplasm—probably macrophages; the cytoplasm is packed with acid-fast bacilli, so that the contours of the cell-body are often invisible. A few giant cells, with several peripheral nuclei, containing numerous bacilli in their cytoplasm are also visible. Some organisms may be found free.

In the *musculo-cutaneous type* the rat is emaciated; the skin presents one or more irregularly round or oval areas of alopecia, commonest on the head; occasionally ulcers are seen, about 0·5 cm. in diameter, covered with a mealy-looking discharge containing acid-fast bacilli. At the site of these areas of alopecia the skin is atrophic. The subcutaneous tissues are devoid of fat, and show the presence of a greyish-white, granular material, which Stefansky regards as altered muscle tissue; it contains numerous acid-fast bacilli. Sometimes nodular masses are found in the muscles, covered with particles of stretched atrophic skin. Stefansky believes that the primary lesion begins in the subcutaneous muscle fibres, and sometimes spreads to the skeletal muscles. Histologically, numerous acid-fast bacilli are found in the corium and subcutaneous tissue; some are free, but most are situated in the cells of the granulation tissue, and in large cells rich in cytoplasm, which resemble the lepra cells of Virchow, but contain no vacuoles. Acid-fast bacilli invade the muscle fibres, collect around the nuclei, and lead to destruction of the tissue. Lesions of the internal organs are rare; but McCoy (1913) states that nephritis is very common; the kidneys are enlarged, yellowish-brown, friable, and often cystic, but no acid-fast bacilli are found in them. In an examination of 186 leprous rats, McCoy (1913) noted alopecia in 47·5 per cent., cutaneous ulcers in 22 per cent., diffuse subcutaneous infiltration in 97·9 per cent., enlarged lymphatic glands in 87 per cent., and nephritis in 53·8 per cent.

Inside the cells the bacilli are said to be distributed not in cigar-like bundles like the human leprosy bacillus, but at random like pins in a packet; and, instead of displacing the nucleus, they arrange themselves round it (Marchoux 1933).

As is clear from the observations of Lampe and de Moor quoted above, the disease may often be present in a latent form.

The **bacteriology** of the disease has already been discussed in Chapter 16.

Reproduction of the Disease in Animals.—Stefansky failed to infect rats or guinea-pigs, but Dean (1905) was more successful. He inoculated 30 rats with suspensions of infiltrated skin and enlarged lymphatic glands. Many of the animals died of intercurrent disease, but 9 of them developed marked lesions. In 3 cases the

infection was passed in series from one rat to another, and from this to a third. The experimental disease ran a slow course, lasting from 6 months to a year. Post mortem, after subcutaneous inoculation, there was sometimes a local lesion containing semi-caseous material and acid-fast bacilli; in these cases the regional lymphatic glands were sometimes invaded by bacilli. After intraperitoneal inoculation the lesions were more extensive: the epiploon was infiltrated with grey masses of opaque material, which consisted of collections of cells containing bacilli. The liver was not usually involved, but in some animals there were small, pale, sharply circumscribed areas, composed chiefly of acid-fast bacilli. The capsule of the spleen was often thickened and contained bacilli, but in only one case was the organ itself involved. In 3 animals there was a tumour-like mass in the anterior mediastinum, consisting of pale-yellow material, which contained large numbers of bacilli. Small nodules in the lungs were found in two cases. The organism proved non-pathogenic to guinea-pigs, rabbits, mice, and a macaque monkey.

The way in which natural infection occurs is not known, but the presence of lesions in the skin and subcutaneous tissues suggests that it is contracted through cutaneous fissures and abrasions. According to Marchoux (1933), the disease can be reproduced by scarification of the skin; and Marchoux and Chorine (1938) state that the subcutaneous inoculation of as few as 5 bacilli will suffice to infect the rat. Wayson and Masunaga (1935), however, suggest that infection may occur through the nose. Examination of wild rats affected with leprosy, and of white rats inoculated subcutaneously, revealed the presence of acid-fast bacilli in the nasal mucosa. Moreover, nasal instillation of a lepromatous suspension was followed by development of rat leprosy. Against this suggestion is the fact that in the natural disease the bacilli seem to be commonest in the inguinal and axillary rather than in the cervical glands. The same objection applies to conjunctival infection, which Marchoux and his colleagues (1935) have shown to be successful under experimental conditions. Further observations are clearly desirable.

Sellards and Pinkerton (1938) have shown that progressive lesions can be readily reproduced by the intracerebral inoculation with rat leprosy bacilli of rats and mice, less readily of monkeys (*M. rhesus*) and rabbits, but not of guinea-pigs. In mice the infection can be reproduced serially by simple intraperitoneal inoculation.

Balfour-Jones (1937) found that the disease could be transmitted to the Syrian hamster (*Cricetus auratus*). Intraperitoneal inoculation with 0·25 ml. of a suspension of infected material from a rat caused death in about 6 months. The picture at post mortem was similar to that in rats, except that the omentum was less thickened. Lesions were commonly present in the liver and spleen, less often in the kidneys and lungs. When interstitial nephritis was observed, clumps of acid-fast bacilli were found in the interstitial tissue. In rats, it may be noted, acid-fast bacilli are never found in the kidney. Subcutaneous inoculation also produced lesions in the hamster.

JOHNE'S DISEASE

SYNONYMS.—*Enteritis chronica pseudotuberculosa bovis; Entérite spécifique chronique des bœufs; Chronischer infektiöser Darmkatarrh des Rindes;* chronic bovine pseudo-tuberculous enteritis.

Johne's disease is a specific enteritis affecting cattle, and less frequently sheep and deer, caused by an acid-fast organism known as Johne's bacillus. It was first described by Johne and Frothingham in 1895 at Dresden. They regarded it as a peculiar form of tuberculosis, possibly due to the avian type of bacillus.

The disease is fairly common in Northern Europe, and has been observed in America. It affects particularly young cows, but no age is exempt. Since, however, the incubation period is lengthy—up to about 18 months—it is uncommon to meet with the disease in cows less than 2 years old. Latent infection is apparently quite frequent. Sheep are less often attacked, though according to McEwen (1939) the disease may exact an annual toll of 5 per cent. of the animals. In this country the disease is prevalent in cattle, especially those on marshy or boggy land. Natural infection probably occurs through the ingestion of fodder that has been soiled with fæces of infected animals. It has been shown experimentally that Johne's bacillus may remain alive for several months in infected fæces and ditch water exposed to ordinary atmospheric conditions (Lovell, Levi and Francis 1944).

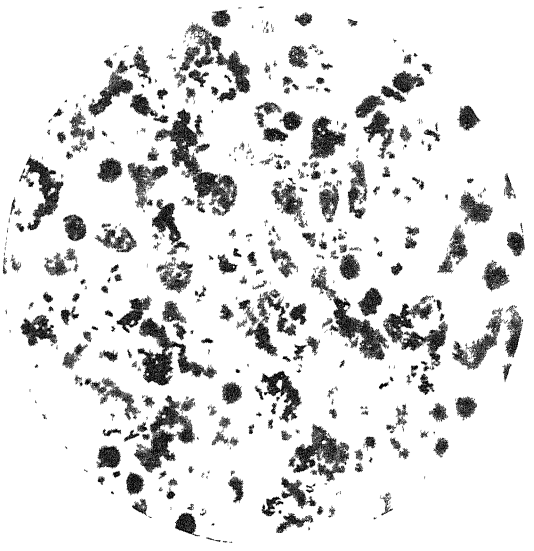


FIG. 281.

Section of intestine of cow with Johne's disease. The bacilli are seen in dense clumps, mostly intracellular (× 1000).

Clinically the disease runs a chronic course, subject often to long periods of intermission, and characterized by unthriftiness, a falling off in the milk yield, diarrhœa of varying severity, and progressive emaciation; there is no fever.

Pathologically, the main lesions are situated in the last 50 feet of the small intestine, and in the neighbourhood of the ileocæcal junction, but sometimes the whole gut is affected. The intestine is thick and rigid, looking not unlike a hose pipe; the mucosa is greatly thickened and is thrown into regular corrugations, resembling the convolutions of the cerebrum. The mucosa is smooth and pale or pink in colour, and covered with slimy material; on the surface of the corrugations it is dotted with red spots or hæmorrhages; between the folds it often has a warty appearance. Occasionally small nodules are seen, due to enlargement of solitary lymphoid follicles. There is no ulceration of the mucosa, and the peritoneal surface appears normal. The mesenteric lymph glands are usually enlarged, œdematous and pigmented. Histologically, the mucosa, and to a less extent the submucosa, are infiltrated with lymphoid and epithelioid cells, which are responsible for the thickening. Near the surface of the gut the mucosa is absolutely structureless; all traces of nuclei and cell outlines have disappeared. Giant cells are rare; there is no caseation, ulceration, fibrosis, or calcification. Short acid-fast bacilli, often in enormous numbers, are found in the mucosa lying between the glands and in the lymphoid tissue of the solitary follicles; they may invade the submucosa, and very occasionally the underlying muscle layer. The bacilli are

arranged in dense clumps, and may be intra- or extracellular. Johne and Frothingham were of the opinion that they were primarily intracellular in position, but that owing to the subsequent disintegration of the cell, they were set free in the tissue. The cellular reaction around the bacilli is diffuse, not localized as in tuberculosis. Bacilli are generally found in the mesenteric glands and sometimes in the sublingual and submaxillary glands. In sheep there may be very much less thickening and corrugation of the intestinal mucosa than in cattle. The disease can be reproduced in cattle and sheep by feeding or parenteral inoculation with pure cultures of Johne's bacillus (see Chapter 16).

The **bacteriology** of the disease has already been described in Chapter 16.

Diagnosis and Prophylaxis.—Differential diagnosis has to be made from strongylosis, coccidiosis, and particularly tuberculous enteritis. The tuberculin test with bovine tuberculin is negative, but a reaction often follows the injection of avian tuberculin (see Bang 1909, 1914). Twort and Ingram (1913) advocate the use of a preparation like tuberculin, but made from glycerine broth cultures of Johne's bacillus. M'Fadyean, Sheather and Edwards (1912) prepared a similar substance which they termed *johnin*. These products, however, do not appear to be strictly specific; reactions may be obtained in tuberculous animals. Dunkin (1928) and Minett (1933) have recorded successful results with johnin prepared by growing Johne's bacillus in a special broth medium containing dead *phlei* bacilli, and more recently (Dunkin 1933) with johnin prepared from cultures in a synthetic medium free from extracts of acid-fast bacilli. This product is said not to give rise to the non-specific swellings that sometimes follow the use of johnin made from phlei-containing media. McEwen (1939), however, found the johnin test unreliable both in sheep and cattle. His experience seems to be borne out by the results of a comparative trial conducted under the auspices of the Agricultural Research Council (Report 1941). The johnin was prepared from cultures in a synthetic medium. Two samples were used, one prepared by heat concentration, the other by precipitation. The double intradermal test was made on 103 cattle. There was considerable disagreement (*a*) between the readings of different observers, and (*b*) between the results of the skin test and the presence of Johne infection as revealed by post-mortem examination. In only 19 out of 52 reactors could infection be demonstrated at necropsy; on the other hand it was found in 6 out of 17 negative reactors. The reason for these discrepancies was not ascertained. A P.P.D. preparation of johnin, corresponding to the precipitated protein derivative of tuberculin (see p. 1328), has been described, but so far no records are available of its use in the field (McIntosh and Konst 1943).

The bacilli are sometimes found throughout the intestine; in such cases diagnosis is often possible by microscopical examination of a piece of rectal mucosa removed with forceps or a Volkmann's spoon. The bacilli are sometimes found in the fæces, generally in very small numbers (M'Fadyean *et al.* 1912); but it is impossible without cultural examination to distinguish them from saprophytic acid-fast bacilli. In any case in which acid-fast bacilli are found, it is advisable to inject them into a guinea-pig, in order to exclude the possibility of their being tubercle bacilli. Agglutination and complement fixation are said to be of little value in distinguishing Johne's bacillus from the tubercle bacillus (Twort 1912).

The disease is usually fatal, but numerous instances of apparent recovery or of long intermissions in its course are known. In some of these animals post-mortem examination has revealed the presence of intense cellular reactions in the mesenteric

glands, which had apparently resulted in the disappearance of the bacilli. It seems probable that in a proportion of animals infection remains latent and never gives rise to clinical symptoms.

Since the disease appears to be spread mainly by the contamination of water, food-stuffs and pasture land with the fæces of infected animals, preventive measures will include the destruction of diseased animals, suitable disposal of their excreta, disinfection of byres, and the ploughing-up of infected pasture. If this is impracticable, pasture land should be regarded as potentially dangerous for at least a year and should not be used for the grazing of sheep or cattle. These measures alone will not suffice to eradicate the disease. A more hopeful policy might be to test all members of the herd with johnin, to segregate and ultimately remove the reactors, to disinfect stalls and buildings, and to raise the non-infected animals on clean pastures (Hutyra and Marek 1926, Minett 1933); but until the johnin test itself can be made more reliable than it is at present, it is doubtful how far such a policy is likely to be successful.

According to Vallée, Rinjard, and Vallée (1934), subcutaneous vaccination of non-infected animals with 5–10 mgm. of living virulent cultures of Johne's bacillus affords a high degree of protection against the disease. This statement awaits confirmation.

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CHAPTER 61

DIPHTHERIA AND OTHER DISEASES DUE TO CORYNEBACTERIA

THE modern conception of diphtheria as a clinical syndrome may be regarded as dating from the observations of Bretonneau in 1826. Its conception as a disease with a single bacterial cause dates from the publication of Loeffler's classical paper in 1884. Klebs had described the bacillus in the diphtheritic membrane in the previous year, but he had brought forward no convincing evidence of its ætiological rôle. The best possible introduction to a discussion of the bacteriological aspects of diphtheria is a brief summary of Loeffler's experiments. He demonstrated the presence of the bacillus, which we now know as *C. diphtheriæ*, in the throats of 13 of 22 persons suffering from clinical diphtheria. From 6 of these cases he isolated the organism in pure culture. With these strains, and with remote subcultures from them, he inoculated mice, rats, guinea-pigs, rabbits, finches, pigeons, fowls and monkeys. The mice and rats proved completely resistant. The guinea-pigs died in 2 to 5 days, after subcutaneous inoculation, showing at necropsy a localized area of hæmorrhagic œdema at the site of inoculation, pleural effusions which were often blood-stained, areas of pulmonary consolidation, and marked congestion of the suprarenals. The small birds died in every case within a few days, showing at necropsy an area of œdema, hæmorrhage and necrosis in the breast muscles, where the inoculation had been made. Many of the rabbits were inoculated into the trachea, or into the cornea ; in several of these typical false membranes developed. Similar false membranes were produced by intratracheal inoculation in pigeons. The introduction of cultures of *C. diphtheriæ* into the vagina of guinea-pigs, after minimal injury of the mucosa, was also followed in some cases by typical membrane formation, with local œdema ; and sometimes by generalized symptoms terminating in death. Attempts to produce infection without injury to surface epithelium were not, however, successful. The simple application of cultures of virulent bacilli to the mucosa of the nose or throat, or to the conjunctiva, of rabbits or guinea-pigs was without result ; nor did a monkey whose throat was vigorously brushed with a living culture develop more than a moderate inflammatory lesion which healed in a few days. Attempts to infect rabbits, guinea-pigs, fowls and pigeons by exposing them to a spray of living culture were unsuccessful. No clear case of paralysis, similar to that encountered as a sequela of human diphtheria, was observed in any of these animals. One highly significant point was noted ; in most of those animals which died the bacilli could be recovered only from the tissues in the neighbourhood of the site of infection. Cultures taken from the lesions in the internal organs or tissues usually remained sterile. Loeffler concluded that death was due not to the dissemination of bacilli throughout the body, but to the effect of some substance elaborated in the local lesion.

Thus Loeffler had shown that, in certain animals and under suitable conditions,

the bacillus which he had isolated would produce lesions closely resembling those of human diphtheria. This demonstration was the logical extension of the results recorded by Trendelenburg in 1869 and by Oertel in 1871, both of whom had succeeded in producing false membranes in rabbits and pigeons by inoculating them with material from human lesions. He had further shown that the subcutaneous or intramuscular inoculation of cultures of the bacillus into susceptible animals was followed by death associated with lesions which, while they bore little superficial resemblance to human diphtheria, were in themselves highly characteristic; that these lesions were not the direct result of tissue invasion by the bacilli: and that certain animal species were highly resistant to infection by any route.

The next important step in our knowledge of diphtheritic infection was the discovery by Roux and Yersin in 1888 that sterile filtrates from cultures of the diphtheria bacillus would kill guinea-pigs with lesions identical with those which follow the injection of the living organism. This demonstration of the presence of a potent extracellular toxin afforded an explanation of Loeffler's observation that death was not associated with any spread of bacteria beyond the local lesion. In rabbits inoculated with such filtered cultures Roux and Yersin noted the occurrence of late paralysis.

Later investigations have confirmed and extended these pioneer observations, and we now have a fairly detailed knowledge of the reaction of a large number of animal species to the diphtheria bacillus and to its toxin.

The lesions produced in experimental animals have been described on pp. 457 and 458. We are here concerned with them only in so far as they throw light on the pathogenesis of diphtheria in man. The epidemiology of diphtheria, the way in which the disease attacks herds as opposed to individuals, will be more conveniently considered in a later section of the chapter, when we have discussed the diagnostic methods on which our present knowledge largely depends.

It will be recalled that experimental diphtheritic toxæmia in the guinea-pig is associated with fatty degeneration of the heart muscle and diaphragm, and with congestion and hæmorrhage in the adrenal glands. There can be little doubt (see Bolton 1905, Dudgeon 1906, Jaffé 1920, Andrewes *et al.* 1923) that the direct action of diphtheria toxin on the heart muscle is one of the most important causes of death in human diphtheria.

With regard to the adrenal lesions, it may be noted that the gross changes, which are so characteristic of diphtheritic toxæmia in the guinea-pig, are not found in fatal cases in man. There is, however, a growing body of evidence which suggests that suprarenal congestion, with small extravasations of blood, and degenerative changes in the cortical or medullary cells, is of frequent occurrence in fatal human infections; though these lesions are revealed only by careful histological examination (Andrewes *et al.* 1923). So far as the nervous lesions are concerned we need only indicate the obvious analogy between the late paralysis of experimental diphtheria in the rabbit, guinea-pig or dog, and the characteristic paralysis in man.

We may, then, regard diphtheria as an infection in which a characteristic local lesion, the membranous inflammation of the fauces, is associated with an equally characteristic toxæmia, in which a powerful exotoxin exerts a selective poisonous action on certain groups of cells.

We have already referred to Loeffler's observation, that the diphtheria bacillus can be recovered from the local experimental lesion, but not usually from the

internal organs. These findings have been generally confirmed by subsequent investigators, though a few discordant results have been recorded (Andrewes *et al.* 1923). In human diphtheria it would appear that some degree of tissue invasion is of fairly frequent occurrence, though *C. diphtheriæ* has very rarely been isolated from the blood during life; Andrewes and his colleagues (1923) note 3 positive results in the records of 313 cases studied by Leede, Roedelius, and Reiche in Hamburg. The bacteriological findings at autopsy are somewhat discordant, and difficult to interpret. That *C. diphtheriæ* has, on occasion, been cultivated from most of the internal organs examined there is little doubt; but the technique employed has varied widely; and, with the single exception of the general agreement that the diphtheria bacillus may be isolated from the lungs in a considerable proportion of cases of fatal diphtheria, there is little agreement as to the frequency with which it may be found in the various organs and tissues. As Andrewes and his colleagues point out, the presence of *C. diphtheriæ* in the lungs may well be due to direct inhalation, rather than to active spread. The evidence, as a whole, clearly suggests that natural diphtheria in man, like experimental diphtheria in the guinea-pig, rabbit, or pigeon, is to be regarded as essentially a toxæmia, in which tissue invasion is minimal; and in which individual resistance or susceptibility to infection, and recovery or death when infected, are largely determined by the presence or rapid production of antitoxin.

This does not mean that invasiveness, as a character of the bacterium, plays no part in the natural or experimental disease. Where the *materies morbi* consists of living bacilli, some degree of local tissue invasion and bacterial proliferation must occur if a significant amount of toxin is to be elaborated and absorbed. It follows that the virulence of a particular strain of *C. diphtheriæ* is not completely defined by determining its toxigenicity.

That this is so has been demonstrated very clearly by recent observations on the case-fatality rates associated with infections caused by different types of *C. diphtheriæ*.

The Gravis, Mitis and Intermediate Types of C. diphtheriæ in Relation to Diphtheria in Man.

We have already referred (pp. 450, 462) to the description by McLeod and his colleagues (Anderson, Happold, McLeod and Thomson 1931) of three different types of *C. diphtheriæ*, and have noted that the *gravis* and *intermedius* types are associated with a high case-fatality rate, while infections caused by the *mitis* type are usually far milder.

The differences recorded are very striking. For example, the incidence of complications varies both qualitatively and quantitatively. Table 110, taken from Cooper and his colleagues (1936), makes this clear.

TABLE 110

COMPLICATIONS AND DEATHS ASSOCIATED WITH DIFFERENT TYPES OF DIPHTHERIA BACILLI.

Type.	Total Cases.	Hæmorrhagic. Per cent.	Paralytic. Per cent.	Laryngeal. Per cent.
Gravis	2,313	3·5	17·0	2·3
Intermedius . . .	1,993	3·7	9·9	1·3
Mitis	1,488	0·4	4·5	7·5

It would appear that paralytic complications are commonest in *gravis* infections, and obstructive lesions in the air passages in *mitis* infections, and that hæmorrhagic complications are far more frequent in infections due to the *gravis* or *intermedius* than to the *mitis* type. So far as albuminuria is concerned, Anderson and his colleagues (1933*a*) record a 52·6 per cent. incidence among their *gravis* cases, 52·1 per cent. among their *intermedius* cases, and 28·4 per cent. among their *mitis* cases.

There are also considerable differences in the case-fatality rate associated with the different types. McLeod (1943), who has collected data comprising about 25,000 cases from different parts of the world, gives the following figures:—

8·1 per cent. in 11,492 *gravis* cases.
7·2 „ „ „ 6,807 *intermedius* cases.
2·6 „ „ „ 6,858 *mitis* cases.

The sharp difference between the severity of the *mitis* cases and those due to infections with the *gravis* and *intermedius* types emerges clearly from the available records. It is much less clear that there is any consistent difference between the severity of *gravis* and *intermedius* infections. There is at least a suggestion (Robinson and Marshall 1934, 1935) that whichever of these two types assumes temporary dominance during a particular epidemic prevalence gives rise to the higher case-fatality rate.

All these figures, it should be noted, refer to cases treated with antitoxin. It would seem to be established that, under these conditions, the mortality associated with *mitis* infections is usually low. Variations, however, occur in different places and in different epidemics. According to Cooper and his colleagues (1936), all three types may be met with in phases of exalted and of diminished virulence, the *gravis* at its lowest corresponding to the *mitis* at its highest, and the *intermedius* at its highest surpassing the *gravis* in its lower or moderate phases of virulence.

That wide variations occur in the frequency of the different types at different times and in different places has been clearly established. Thus, Anderson, Cooper, Happold and McLeod (1933*a*) record the following frequencies in Leeds: *gravis* infections 75·62 per cent., *intermedius* infections 4·27 per cent., *mitis* infections 20·11 per cent. Later information is afforded by Cooper and his colleagues (1936) and by W. T. Russell (1943), both of whom analyse the distribution of the types in several different centres in England and Wales, and by McLeod (1943), who includes data from many different countries. In general, the predominant strain is far more often of the *gravis* or *intermedius* than of the *mitis* type, suggesting that *mitis* diphtheria is usually endemic, whereas *gravis*, and to a less extent *intermedius*, diphtheria tend to be epidemic. Changes in distribution of the different types may occur from year to year (see Robinson and Marshall 1934, Wright 1941*b*, Report 1943). For example, in Manchester the *gravis* infections rose from 23·8 per cent. in 1934 to 50·9 per cent. in 1935; in Liverpool they rose from 34·2 per cent. in 1937 to 69·6 per cent. in 1940. It may be noted that in the United States some workers have failed to type their strains satisfactorily; the reasons for this cannot be discussed here (see Perry *et al.* 1936, Frobisher 1938, 1940, 1942, Seligmann 1941).

Closely bound up with the distribution and case fatality of the different types is their capacity to give rise to clinical diphtheria in naturally Schick-negative or immunized persons. Several observations have been made on this subject. From McLeod's (1943) review it seems clear that, apart from the experience of Liverpool, where *intermedius* infections have been particularly severe, diphtheria in the immunized population has been due mainly in the past to infections with the *gravis* type. There is evidence, too, to show that in a well-immunized population diphtheria may be practically absent so long as the *mitis* type is predominant,

but that numerous cases may occur if the *gravis* type is introduced and gains the ascendancy.

The evidence at present available does not allow of any definite conclusion in regard to the factors that determine the greater virulence of *gravis* and *intermedius* as compared with *mitis* strains. Early observations (see Parish, Whatley and O'Brien 1932*a*, *b*, Anderson *et al.* 1933*a*) showed that all three types were highly toxigenic, and that the toxins appeared to be identical in respect of their neutralizability by antitoxin. Similarly no obvious differences were apparent in the virulence of the three different types for guinea-pigs. Later observations, however, have tended to reveal small but possibly significant differences between them.

Povitsky, Eisner and Jackson (1933), for example, found the minimal lethal dose of *gravis* strains for the guinea-pig to be smaller than that of *mitis* strains; they also found that antitoxin was rather more effective in controlling *mitis* than *gravis* infections. Murray (1935) reported that it was more difficult to protect rabbits with standard antitoxin against repeated doses of *gravis* bacilli than against similar doses of the other types. Gundel and König (1938) observed that, for protecting animals by active immunization against *gravis* strains a qualitatively better antigen was required than against *mitis* strains. Gundel and Erzin (1936) noticed differences in guinea-pigs infected with standard doses of the three types; those infected with *gravis* strains died soonest, and those infected with *gravis* or *intermedius* strains yielded positive cultures from the internal organs more frequently than those infected with *mitis* strains. Finally, Clauberg (1939), after examining a large number of toxins grown under standard conditions, reached the conclusion that *gravis* toxins were more potent in producing a skin reaction than *mitis* toxins and that their neutralization required more antitoxin.

This evidence, on the whole, suggests that *gravis* strains under appropriate conditions produce a slightly more potent toxin and are more invasive than *mitis* strains.

Some observers have noted a relation between the iron content of synthetic media and the potency of the toxin produced (Pappenheimer and Johnson 1936, Happold 1940); and Mueller (1941) has put forward the interesting suggestion that in the human body, where iron is abundant, *gravis* strains may be the most potent toxin producers. Zinnemann (1943), however, who grew strains of different types in a broth medium having an iron concentration similar to that in the human tissues and tested the minimal reacting dose by intracutaneous inoculation of guinea-pigs, obtained no evidence to support this suggestion.

O'Meara (1940) offers a different explanation. He finds that, if a bacteria-free saline extract of a *gravis* strain is inoculated subcutaneously into a guinea-pig along with a dose of the classical Park-Williams 8 (*mitis*) toxin, the M.L.D. of the toxin is not affected, but there is a greatly increased tissue reaction at the site of inoculation as evidenced by extensive œdema and necrosis. He postulates, therefore, the existence in *gravis* toxins of two factors, A and B, only one of which, A, is present in any considerable proportion in Park-Williams 8 toxin. This may account for the greater seriousness of *gravis* diphtheria in human beings and for the inability of commercial antiserum, which is made chiefly from Park-Williams 8 toxin, to neutralize completely both toxic factors produced by strains of the *gravis* type. No confirmation is yet available of O'Meara's work, and further observations will be required to test the truth of his explanation.

The Diagnosis of Infection in Diphtheria.

The laboratory diagnosis of infection with diphtheria depends on the demonstration of the causative organism. Though we are concerned here more with general principles than with technical details, it will be convenient to describe the current practice in most British laboratories. In the past chief reliance was placed on examination of a direct smear, and on the microscopic appearance of the growth of a Loeffler slope culture. Since the differentiation by McLeod and his colleagues (Anderson *et al.* 1931, 1933*a*, *b*) of three different colonial types of diphtheria bacilli on tellurite blood agar, and since the agreement reached by numerous workers on the advantage of using a selective medium, the study of the colonies developing on tellurite plates has assumed increasing importance in the laboratory diagnosis of diphtheria. Not only does the isolation of diphtheria bacilli in the form of single colonies carry greater assurance than their demonstration morphologically in a film containing numerous other organisms, but the recognition of their type—*gravis*, *intermedius*, or *mitis*—proves often of epidemiological and sometimes of clinical interest. We may outline the general procedure to be followed and the interpretation of the results in looking for the diphtheria bacillus in suspected cases of the disease, and in convalescents, contacts, and suspected carriers.

Suspected Cases of Clinical Diphtheria.

By examination of a smear made directly from the swab and stained preferably by the wet-film technique, it is sometimes possible to make a presumptive diagnosis of diphtheria. Though this method is advocated by a few workers, the majority of bacteriologists do not regard it with favour. It is restricted in its usefulness to throat swabs from children, and even in these the proportion of positive results in true cases of diphtheria is often fairly low. Though it should not do so, a negative result serves as an excuse to many practitioners to withhold serum for treatment. A preliminary negative report on a direct smear has frequently to be followed by a positive report on culture, thus confusing the practitioner and leading him to doubt the accuracy of the laboratory's findings. Moreover, the time taken in preparing and examining direct smears may often be spent more profitably in other ways. Without denying the value of the method in special cases, most bacteriologists are agreed that for routine work it is much better abandoned. In some laboratories a film is made from throat swabs and stained for the characteristic spirilla and fusiform bacilli of Vincent's angina. If these organisms are found microscopically in the cultural absence of diphtheria bacilli and of hæmolytic streptococci, they may afford an explanation of the clinical condition of the patient, and may be reported without prejudicing his treatment.

In the routine *cultivation* of swabs from suspected cases of diphtheria it is advisable to inoculate each swab on to a slope of Loeffler's serum, a plate of tellurite blood agar, and, for the detection of hæmolytic streptococci, on to an ordinary blood agar plate. All three cultures should be examined 18–24 hours later. Positive results obtained on both Loeffler and tellurite, or on tellurite alone, can be reported safely at once. Characteristic findings on Loeffler in the presence of a negative tellurite plate usually justify a presumptive positive report. When, however, the results are ambiguous or negative, it is better to send an interim doubtful report, and await the result of subculture or of further incubation of the tellurite plate before a final negative report is made. In cases of this sort

the finding of Vincent's spirilla and fusiform bacilli in direct film or of hæmolytic streptococci on a blood agar plate is often of value in suggesting the true nature of the infection.

This is not the place to discuss the relative merits of the various tellurite blood agar media that have been proposed. In different laboratories preference is expressed for the particular medium described by McLeod and his co-workers (Anderson *et al.* 1931), by Clauberg (1929, 1931, 1933, 1935, 1936), by Horgan and Marshall (1932), by Neill (1937), by Glass (1937), by Hoyle (1941), or by some other worker. So far, no medium can be regarded as perfect. We have given our reasons already (p. 451) for preferring a tellurite agar containing unheated and unlysed blood. Whatever medium is used, however, there will always be a small proportion of colonies that cannot be recognized with certainty within 24 hours; a final negative report should therefore not be issued for 2 days after receipt of the swab.

The combined use of a Loeffler slope and a tellurite blood agar culture enables a high proportion of all cases of diphtheria to be diagnosed bacteriologically within 18–24 hours. Early investigators, such as Weigmann and Degn (1932), Lewis (1933), Kairies (1933), Kemkes (1934), and Hasenbach (1935) found tellurite medium to give a slightly higher proportion of true positive results than Loeffler's serum. The experience of numerous workers has since confirmed their results, and has shown that in *cases* of diphtheria the causative bacillus may be demonstrated by tellurite in about 5–10 per cent. more swabs than by Loeffler. The routine use of a Loeffler slope culture has, however, numerous advantages. It shows whether the swab was suitable for examination; failure of growth suggests that the patient's throat had been treated recently with an antiseptic, or that the swab had been improperly taken or had been too long in transmission to the laboratory. Development of diphtheria bacilli is rather more rapid on Loeffler than on tellurite, and a tentative diagnosis can therefore sometimes be made earlier. Occasionally strains of diphtheria bacilli are met with that are unusually sensitive to potassium tellurite, and may not develop on primary culture in the presence of this substance; to avoid missing these, a Loeffler serum culture is a valuable safeguard. On the other hand, the presence of apparently typical diphtheria bacilli on Loeffler in the complete absence of diphtheria colonies on tellurite must always be regarded with suspicion, and a final positive report should never be sent till diphtheria bacilli have been isolated in pure culture by plating the Loeffler slope growth on to chocolate blood agar or tellurite blood agar.

The routine use of a tellurite blood agar plate is of value partly because it increases the proportion of positive results, and partly because it enables the type of the infecting organism to be determined. By careful examination of the colonial form under a binocular dissecting microscope and of the morphology of the organisms in a wet-stained film, or even by nigrosin (Fleming 1941), many workers of experience can assign correctly the majority of cultures to the *gravis*, *intermedius*, or *mitis* types; but it is always wise to confirm this preliminary identification by means of fermentation and, if necessary, virulence tests.

Shone, Tucker, Glass, and Wright (1939), who made a very careful study at Liverpool of the comparative merits of Loeffler and tellurite in the diagnosis of diphtheria cases, found that of 1,501 strains of diphtheria bacilli 92·4 per cent. were recognized on both a Loeffler slope and a tellurite blood agar plate, 2·3 per cent. on Loeffler only, and 5·3 per cent. on tellurite only. The failures of the Loeffler medium seemed to be due partly to the scantiness of the diphtheria bacilli

in some cases and the consequent difficulty of finding them among other bacteria, and partly to the atypical appearance of certain strains, especially those of the *gravis* type. The chief disadvantage of the tellurite medium lay in the slow growth of some strains of the *mitis* type. Cooper and his colleagues (1940), who made a similar series of observations in McLeod's laboratory at Leeds, found that a tellurite blood agar plate yielded at least 10 per cent. more positive results than a Loeffler slope, and led to less likelihood of missing severe cases. On Loeffler the very short form of *intermedius* type of diphtheria bacillus was found to be difficult to distinguish from unusually granular Hofmann strains. The results on Loeffler's medium at Liverpool were more favourable than those obtained in many other laboratories, probably because Wright and his colleagues had spent two years' intensive work checking the accuracy of their microscopical diagnosis. In the hands of less skilled observers the proportion of true positive results on Loeffler would have been lower, and a number of false positives would almost certainly have been recorded (see Cruickshank 1943).

Convalescents, Contacts, and Carriers.

In swabs from convalescents, contacts, and carriers diphtheria bacilli are generally fewer than in those from cases. The use of tellurite medium in their detection is not only an advantage, it is almost imperative. Numerous workers have found that approximately twice as many positive results are obtained on this medium as on Loeffler's serum. The development of the colonies is, however, often slow, and a negative report should rarely be made till the plates have been incubated for 48 hours.

Virulence Tests.

At one time the only way to ascertain whether a strain of diphtheria bacillus was virulent, and therefore potentially infective, was by inoculating it into a guinea-pig. Though this is still true if complete assurance is to be obtained, the number of virulence tests to be carried out may be greatly reduced by noting the type of diphtheria bacillus to which the strain belongs. Considerable experience now in many laboratories has shown that nearly all strains of the *gravis* and *intermedius* type are virulent, but that some strains of the *mitis* type are avirulent. Thus Parish (1936) reports 96·9 per cent., 99·5 per cent., and 99·7 per cent. of virulent cultures among large samples of *gravis* strains examined by different workers, and 94·1 per cent., 99·1 per cent., and 99·9 per cent. for *intermedius* strains, as against 80·0 per cent., 86·6 per cent., and 87·5 per cent. for strains of *mitis* type. Stallybrass (1936), quoting Wright's findings in Liverpool, reports one avirulent culture among 145 *gravis* strains, 4 avirulent cultures among 116 *intermedius* strains, but no less than 100 avirulent cultures among 231 *mitis* strains. The proportion of avirulent strains varies also with the origin of the culture. Thus strains of all three types are invariably virulent when isolated from cases of acute diphtheria. Avirulent strains are encountered among convalescents, contacts, and carriers, particularly in those with nasal and other extra-faucial infections. Strains of the *gravis* and *intermedius* type need seldom be tested for virulence unless derived from long-standing carriers. Strains of the *mitis* type from contacts, carriers, and persistently positive convalescent cases will generally require testing. The strains, however, that most require testing for virulence are atypical strains, especially those found in carriers (Cooper *et al.* 1940).

Two methods of performing a virulence test are in use—the subcutaneous and the

intracutaneous. The technique of each test varies with different workers. In the subcutaneous test it is usual to inoculate between an eighth and the whole of an 18-hour Loeffler slope culture in a 6 × $\frac{5}{8}$ in. test-tube into a guinea-pig. The importance of washing off the growth with a suitable suspending medium such as broth rather than with saline, which destroys the organisms, has been stressed by Holt and Wright (1940). These observers likewise recommend the chest as the preferential site of inoculation, since œdema is said to be more noticeable here than in the thigh. To make certain that the strain under test forms the toxin specific to the diphtheria bacillus it is wise to inoculate two guinea-pigs simultaneously, one of which is protected by means of a suitable dose of antitoxin, such as 500 units given intraperitoneally on the previous evening.

The intradermal, or Römer, method has the advantage of allowing several strains to be tested on the same animal. A Loeffler slope culture is washed off with 1 to 4 ml. of broth, depending on the amount of growth, and inoculated intradermally in a quantity of 0·2 ml. into the shaven side of an albino guinea-pig. A known virulent and a known avirulent strain should always be inoculated as controls. To protect the animal from death during the development of the characteristic skin reactions, 20 to 40 units of refined antidiphtheria serum should be injected simultaneously into the peritoneal cavity. A guinea-pig protected by the intraperitoneal injection of 500 units of serum on the previous evening should likewise be inoculated intradermally with the same strains as those used for the test animals. This precaution is desirable especially with atypical strains and with cultures of doubtful purity, so as to prevent confusion being caused by necrotic lesions due to other species of *Corynebacterium* or to staphylococci.

The Diagnosis of Susceptibility or Immunity to Diphtheria.

Modern methods of diphtheria control depend almost as much on distinguishing between the susceptible and resistant members of the human herd as on diagnosing cases and carriers. The technique that first enabled us to do this on an adequate scale was the test introduced by Schick (Michiels and Schick 1913*a*, *b*, Schick 1913). It is performed as follows.

The test toxin is stored in a relatively concentrated solution, and when required for use is diluted so that one Schick dose is contained in 0·2 ml. Part of this diluted toxin is heated to 70° C. for 5 minutes, to serve as a control. Into the flexor surface of one arm 0·2 ml. of the unheated toxin is injected intradermally, care being taken that the injection is made into the substance of the dermis in such a way as to raise a definite bulla. Into the flexor surface of the opposite arm is injected 0·2 ml. of the heated toxin. The reaction to the toxin develops at a varying rate. In some persons a positive reaction is evident after 24 hours, but in others it is not fully developed for a week. From the observations of Downie and his colleagues (Report 1942*a*), the best times to make readings would appear to be the 4th and the 7th days; if only one reading can be made, the 7th day should be selected. The following types of reaction may be observed to the toxin and the control:—

(*a*) The negative reaction—no reaction of any kind in either arm.

(*b*) The positive reaction—no reaction in the control arm.

In the test arm a circumscribed red flush appears generally after 24 to 36 hours, reaching its maximum development on the 4th to 7th day. At this time there is a circular area, 1–2 cm. in diameter, slightly raised above the general surface of the skin. This slowly fades during the following few days or more, leaving an area of brownish pigmentation, with a desquamating epidermis.

(*c*) The pseudo-reaction (negative).—This reaction, which is of the characteristic allergic type, develops equally in both arms during the first 24 hours. It is less sharply circumscribed than the true positive reaction, and fades much more rapidly. By the 4th day it has usually disappeared, leaving some slight degree of reddish or brownish discoloration.

(*d*) The combined reaction (pseudo + positive).

The control arm shows the succession of changes referred to under the pseudo + reaction. The reaction in the test arm is almost indistinguishable from that in the control arm during the first 24 hours. After this time the reaction in the test arm continues to develop, while that in the control arm commences to fade. By the 4th day the difference between the two arms is usually quite distinctive.

The positive and combined reactions are generally regarded as indicative of susceptibility. More precisely they show that antitoxin is either absent from the circulating blood or is present in only very small quantity. At one time it was thought that a positive reaction showed that there was less, and a negative reaction that there was more, than 1/30 A.U. per ml., but further experience has rendered it clear that either reaction may occur over a fairly wide range of antitoxin values (see Jensen 1931, Fraser and Halpern 1935, Leach and Pöch 1935, Parish and Wright 1938, Thelander 1940, Downie *et al.* 1941). A positive reaction may be registered with as much as 1/30 A.U. per ml. and a negative reaction with less than 1/1,000 A.U. In general, however, a positive reaction is uncommon with 1/100 A.U. per ml. or more, and rare with 1/30 A.U. or more.

The Schick test, within its limits, is a quantitative one; and the test dose must therefore be defined, and the test toxin standardized.

The Schick dose was originally defined on an empirical basis as 1/50th of the guinea-pig M.L.D.; but, as Glenny (1925) has pointed out, we are concerned in the Schick test not only with toxicity but with combining power. We want to know how much toxin the subject's tissue fluids can neutralize. The M.L.D. is not a satisfactory unit for this purpose since it is not a measure of combining power; but an adequate degree of toxicity must be ensured, since the dermal reaction depends on the presence of free toxin.

The subject was considered by the Biological Standardization Commission of the League of Nations in 1931 (see Report 1931), and two alternative definitions of the Schick dose were adopted. One reads as follows:

"The Schick test-dose, for injection in a volume of 0·2 ml., shall be that quantity of toxin which, when mixed with 1/750th part or more of an international unit of antitoxin and injected intracutaneously into a normal guinea-pig, causes no local reaction; but, when mixed with 1/1,250th part or less of an international unit and similarly injected, causes a marked reaction of the type of a 'positive' Schick reaction; *provided that* the toxin is such that 1/25th of the Schick test-dose as above determined, without admixture with antitoxin, when injected intracutaneously into a normal guinea-pig, causes a definite local reaction of the type of a 'positive' Schick reaction; but that 1/50th of the Schick test-dose, similarly injected without admixture, causes no local reaction of this type."

In the alternative definition the main clause defines the Schick dose in terms of the M.R.D., and the proviso deals with combining power. Both conditions must be fulfilled under the Regulations (1931) made in Great Britain in accord with the Therapeutic Substances Act (1925).

As a possible alternative to the Schick test, the actual amount of circulating antitoxin in a person's blood may be determined by titration, using the Römer technique. This is clearly a more accurate method, and entails a single withdrawal of blood compared with the intradermal injections and their observation during the following few days. The necessary animal inoculations might be supposed to prevent the adoption of this method as a routine. Jensen (1933*b*), however, who uses rabbits instead of guinea-pigs for the intracutaneous injections, thus increasing the number of tests that can be carried out on a single animal, considers it the method of choice for both convenience and accuracy.

The evidence now available leaves no reasonable doubt that a person who reacts negatively to the Schick test, or who has been shown to possess an equivalent

amount of circulating antitoxin is comparatively immune to all ordinary risks of infection. This evidence has in part been obtained by keeping under observation samples of the general population whose Schick reaction has been determined, in part by observing the behaviour of negative and positive reactors when exposed to high risks of infection. The evidence available under the latter head is more impressive, and more readily obtained, than that under the former, for the simple reason that, where the risk of infection and consequently the incidence of disease are high, the observation of relatively small samples over relatively short periods of time will serve to reveal statistically significant differences, if such exist. This problem will be referred to again in discussing the efficacy of active immunization. It must be made clear, however, that the degree of immunity possessed by Schick-negative reactors is far from absolute, and that, in fact, quite large numbers of cases of diphtheria have been reported in immunized persons many of whom were probably Schick-negative. Such cases are usually due to infection with the *gravis* type of diphtheria bacillus (Cooper *et al.* 1936, McLeod 1943) and seldom prove fatal.

We may note here the single instance of a direct experiment on man which has, so far as we are aware, been placed on record. Guthrie, Marshall and Moss (1921) collected eight volunteers and determined their reaction to the Schick test; four reacted positively, two negatively, and two gave pseudo-reactions which were read as negative. The throat of each of these eight volunteers was swabbed with a virulent culture of *C. diphtheriæ*. The organism established itself on the tonsils of the four positive reactors, and of three of the four negative reactors. The four positive reactors developed clinical diphtheria; the three negative reactors became temporary carriers without showing any sign of the disease.

In discussing the nature and origin of the normal antibodies in Chapter 49, we have already described the observations of Zingher and of others on the results obtained in the application of the Schick test to large samples of the normal population, and have considered the opposing theories of the origin of normal antitoxin as the result of latent infection, or as a phase of normal biochemical ontogeny, in the absence of any specific stimulus; and we have expressed our personal view that it is impossible to account for the observed distribution of natural antitoxic immunity among the population in general, and among certain isolated groups in particular, without assuming that natural immunization plays a highly important rôle.

The Treatment of Diphtheria with Antitoxin.

The nature of antitoxic immunity and the general principles that determine its practical application have been discussed in Chapter 46. We are here concerned with the methods that are employed in the particular case of diphtheria in man, and the available data with regard to their efficacy.

As regards the commercial preparation of antitoxic sera, we need only note that the animal almost universally employed is the horse, and that immunization is usually carried out by the administration of increasing doses of toxic filtrates, administered at intervals of a few days. The initial dose must be minute, 0·01 ml. or thereabouts. The final dose, usually reached within a period of 6 weeks to 2 months, may be very large, 1 litre or more. When a satisfactory titre has been attained the horse is bled from the jugular vein, with strict precautions as regards sterility; the serum, after separation from the clot, is filtered through a coarse

porcelain candle, and 0·3 to 0·5 per cent. tricresol is usually added as a further precaution to maintain sterility (Dean 1908). The average serum so obtained contains between 600 and 800 units of antitoxin per ml.

Apart from its great theoretical interest, the isolation of antitoxin in a state of chemical purity would be an important advance from the practical point of view. This has not yet been achieved, but considerable success has been attained in the concentration of the active constituents (Brieger and Ehrlich 1893, Brieger and Cohn 1893, Brieger and Boer 1896, Pick 1902, Gibson 1905, Banzhaf and Gibson 1907, 1908–9, Banzhaf 1912–13, Andrewes *et al.* 1923). The methods employed, which vary in minor points of technique, depend on the demonstration that the major part of the antitoxin is contained in that fraction of the serum globulin which is precipitated by half saturation with ammonium sulphate, but redissolved when the precipitate is extracted with a saturated solution of sodium chloride. This so-called "pseudoglobulin" fraction can be separated from the euglobulin, and from the serum albumin. The process is accompanied by the loss of an appreciable fraction of the antitoxin originally present in the serum; but the concentration of antitoxin in the finished product may be increased six or eight times as compared with the untreated serum; and there is a relative elimination of non-effective protein material, though the protein content per ml. is appreciably increased during the process of concentration. An even greater degree of purification is now achieved by the use of proteolytic enzymes to digest much of the unwanted protein (Pope 1938, 1939). The resulting *refined diphtheria antitoxin*, as it is called, has been found by Glenny and Llewellyn-Jones (1938) to be absorbed more rapidly and eliminated more slowly in guinea-pigs than the so-called concentrated diphtheria antitoxin, which is the product of precipitation with ammonium sulphate. It has, moreover, the great advantage of giving rise to serum sickness in a much smaller proportion of patients than either crude or concentrated antiserum; probably not more than about 10 per cent. of diphtheria patients develop symptoms after its use, and these are usually mild.

Our object in treatment will obviously be to introduce as much antitoxin as we can, at the earliest possible moment, since we must act on the assumption that the beneficial result of our interference will probably be limited to the neutralization of the toxin which has not yet been firmly anchored to the cells which are susceptible to its action.

In support of this view we may cite such experimental results as those recorded by Glenny and Hopkins (see p. 1031). For the same reason we should give therapeutic injections of antitoxin intravenously, intraperitoneally or intramuscularly, never subcutaneously, since the relatively slow absorption from the subcutaneous tissues will defeat our main objective (see p. 1124).

It is sometimes taught that the administration of antitoxin is without effect after the 5th or 6th day of disease, a dictum based mainly on the figures recorded by Faber (1904), which show a progressive increase in case mortality from 7·1 per cent. in patients receiving antitoxin on the first day of disease, to 17.0 per cent., 21·3 per cent. and 19·9 per cent. among those treated on the 6th, 7th and later days respectively. It has, however, been pointed out that such hospital statistics are of little value, because there is an important element of selection of which they take no account. Cases admitted to hospital on the 5th day or after will, *ipso facto*, include a large number of severe infections; since the delay in admission will in most cases be due either to delayed diagnosis, often resulting from failure to obtain a medical opinion, or to an unwillingness to resort to hospital. In either case those patients who are well on the way to recovery by the 5th or 6th day will never be admitted, while those who are seriously ill will be transferred to hospital, either because a late diagnosis has been arrived at, or because the serious condition of the patient has alarmed his friends.

There is no justification for withholding antitoxin because a case is in an advanced stage, though every effort should be made to ensure that it is given early in the disease.

A system of dosage in common use is that advocated by Park (1921) (see Table 111); but there has been a tendency to increase the amount of antitoxin given in the severer type of case as the result of the experience of recent years, and this tendency seems likely to continue. Thus, Bie (1922), in an admirable review of the evidence in regard to the value of antitoxin treatment in diphtheria, advocates the administration of very large total doses (92,000—170,000 units). Harries (1945) likewise recommends a large single dose, but considers that it should rarely be necessary to give more than 50,000 units, provided part or whole of the dose is given intravenously.

TABLE 111

UNITS OF ANTITOXIN TO BE ADMINISTERED TO CASES OF VARYING GRADES OF SEVERITY. (After Park.)

Age or Weight of Patient.	Mild Cases.	Early Moderate.	Late Moderate and Early Severe.	Severe and Malignant.
Under 2 years. 10–30 lbs. wt.	2,000–3,000	3,000–5,000	5,000–10,000	7,500–10,000
2–15 years. 30–90 lbs. wt.	3,000–4,000	4,000–10,000	10,000–15,000	10,000–20,000
Adults. 90 lbs. or over .	3,000–5,000	5,000–10,000	10,000–20,000	20,000–50,000
Route of administration .	Intramuscular	Intravenous	Intravenous	Intravenous

It might be expected that after some 50 years it would be easy to produce irrefutable statistical evidence of the beneficial effects of the antitoxin treatment of diphtheria in man. The evidence does, in truth, seem decisive to most of us; that it is still possible to bring forward contrary arguments that are not altogether specious is due to the fact that once a strong presumptive case has been made out in favour of a particular therapeutic measure it is not justifiable to continue the period of trial at a risk of human lives.

Of the classical studies from the early days of antitoxin treatment, the only one that affords an entirely satisfactory basis for comparison is that of Fibiger (1898). For a period of one year all cases of diphtheria admitted to hospital were divided into two groups by separating those admitted on alternate days. All cases admitted on one day were given antitoxin, all those admitted on the next day were treated without antitoxin, and so on throughout the period of trial. The results were as follows: of 239 cases treated with antitoxin 8 died, a mortality of 3·5 per cent.; of 245 cases treated without antitoxin 30 died, a mortality of 12·25 per cent. Applying the formula given on p. 987, the difference between the case mortality in the two groups is 8·75 per cent. and the standard error of this difference is 2·445 per cent., so that the odds against the difference being due to random sampling are several thousands to one (see Table 60, p. 985).

Other results recorded during the early and middle 'nineties—comparisons between the case-fatality rates in hospitals in which antitoxin was given and others in which it was not, or between the experience in one hospital before and after the introduction of antitoxin—all pointed in the same direction; and the period of trial ended with the adoption of antitoxin as a routine method of treatment.

If we survey the course of events since that time we find that they strongly support the view that the use of antitoxin has resulted in a significant lowering of

the case-fatality rate; but they would hardly be accepted as demonstrative proof in the absence of the earlier and controlled trials.

There is, for instance, the decline in the case-fatality rate as shown by the returns of the hospitals of the Metropolitan Asylums Board (see Table 112).

TABLE 112

Year.	Case Mortality per cent.	Year.	Case Mortality per cent.
1889	40·7	1909	9·4
1890	33·5	1910	7·8
1891	30·6	1911	8·4
1892	29·3	1912	6·2
1893	30·4	1913	6·2
1894	29·3	1914	7·9
1895	22·8	1915	8·4
1896	21·2	1916	6·8
1897	17·7	1917	6·7
1898	15·4	1918	7·7
1899	13·9	1919	9·3
1900	12·3	1920	8·6
1901	11·1	1921	8·8
1902	11·0	1922	8·7
1903	9·7	1923	6·8
1904	10·0	1924	7·0
1905	8·3	1925	5·0
1906	8·8	1926	4·9
1907	9·6	1927	4·0
1908	9·7		

Antitoxin came into general use about 1895, and the figures suggest that it had produced its full effect on the case-fatality rate about 10 years later; since when this rate has been kept down to a figure of about one-third of that prevailing in pre-antitoxin days. But, if we are disposed to be critical, we shall note that the downward trend had shown itself before the introduction of the antitoxin, and that secular trends in case fatality are known to occur apart altogether from the introduction of new therapeutic measures—as in the classical example of scarlet fever.

There is another factor which would add to our doubt. At about the same time as antitoxin was coming into general use the basis of diagnosis of diphtheria was changing. The introduction of bacteriological methods made it possible to recognize as diphtheria many cases that would earlier have been regarded as non-diphtheritic anginas. For this reason the total of recorded cases would tend to be increased by the inclusion of many mild infections, and the recorded case mortality would automatically fall.

Somewhat better evidence is afforded by the figures for the mortality rates in cases of laryngeal diphtheria, excluding those cases treated by tracheotomy. This gives a series of cases in which the clinical signs were alone sufficient to establish a diagnosis, and in which the infection may be regarded as uniformly serious. In 1894 the mortality in this class of case, in the M.A.B. hospitals, was 62 per cent.; after 1895 it fell, at first rapidly, then less steeply, until in 1910 it was 11·7 per cent. (Andrewes *et al.* 1923). Even here, however, the possible intrusion of other factors, such as changes in the proportion of all laryngeal cases treated by tracheo-

tomy, and in the period at which tracheotomy is performed, make it difficult to assess the real significance of the recorded figures.

In any case, the clear historical evidence of long-period fluctuations in the severity of diphtheria, as of other infective diseases, forbids us to place too great an emphasis on a progressive fall in mortality lasting for a few decades; and, in recent years, there have been signs that the disease is still able to reassert its killing power. Deicher and Agulnik (1927), for instance, give figures for a Berlin hospital and for one of the districts of that city (see Table 113).

TABLE 113

Rudolf Virchow Hospital.				
Year	1923	1924	1925	1926
Total cases	132	140	114	172
Deaths	8	7	10	30
Mortality per cent.	6·1	5·0	8·7	17·4
Alt Berlin.				
Year	1923	1924	1925	1926
Total cases	1,068	1,016	1,109	1,421
Mortality per cent.	7·58	5·9	8·1	11·1

It is tempting to suggest that this change has been caused by an increase in the proportion of cases due to the *gravis* or *intermedius* types. During the period 1930–32, for instance, a severe form of diphtheria was prevalent in Leeds. It was during this epidemic that McLeod and his colleagues differentiated the three types, and we have already noted that the *gravis* cases showed a case mortality of 13·1 per cent., while no deaths occurred among the *mitis* infections. The case-mortality rate for all infections in this series of cases, *gravis*, *intermedius* and *mitis* combined, was 10·3 per cent., a figure that may be compared with those given in Table 112. Similar figures have been quoted from other observers. It is very possible that this explanation is the true one; but, since we have no information with regard to the relative proportion of *gravis*, *intermedius* and *mitis* infections during the earlier years of this century, when the prevailing case mortality of diphtheria was low, we must wait for further experience before granting it definite acceptance.

We are thus left with a mass of statistical data all of which is compatible with the view that the use of antitoxin has had a considerable effect in lowering the mortality from diphtheria, but most of which is indecisive. Taking the general trend of this evidence with Fibiger's early observations and adding to it the decisive results obtained in animal experiments, we have a sound basis for the conclusion that antitoxin provides the best available method of treatment, and that its early administration is the essential factor in controlling the clinical disease. The failure to establish a statistical case that is beyond criticism merely shows how hard it is to assess the real effect of any remedy in any human disease, unless that effect is so dramatic as to be immediately and consistently evident.

In view of recent experience, in Germany, in England and elsewhere, there is, however, an obvious need to re-examine the problem with a view to improving, or adding to, our present therapeutic reagents.

The Standardization of Diphtheria Antitoxin.

We have already referred to the standardization of diphtheria antitoxin, in our discussion of the toxin-antitoxin reaction (pp. 238–239). We noted that the instability of a toxic filtrate renders it quite unsuitable as a standard of reference, and that, for this reason among others, Ehrlich's original definition of the unit of antitoxin (A.U.) as *the smallest amount of antitoxin that will neutralize 100 M.L.D. of toxin*, using the guinea-pig as the test animal, had soon to be abandoned, and has been replaced by a unit defined in terms of a standard antitoxin. Such a standard antitoxic serum, when dried and preserved *in vacuo* in the presence of phosphorus pentoxide, maintains its potency over long periods of time. It has, moreover, become a general principle in biological standardization that a reagent shall, wherever possible, be assayed by comparing its potency with that of a standard preparation of the same reagent, to which some unit value has been assigned by international agreement. In the case of diphtheria, Ehrlich's original antitoxin was adopted as the international standard. The correct definition of a unit of diphtheria antitoxin is as follows.

One unit of diphtheria antitoxin (1 *A.U.*) *is contained in that amount of an antitoxic serum that has the same total combining capacity, for toxin and toxoid, as one unit of the standard antitoxin.*

Standard antitoxin is now preserved and issued by selected Institutes, and is tested from time to time under the auspices of the Biological Standardization Commission of the League of Nations (see Report 1923).

The methods employed in measuring the potency of an antitoxic serum, and the knowledge that has been acquired in their development and use, have played so large a part in the evolution of our present methods of controlling the disease that, at the cost of some repetition, it is desirable to summarize them here. Three such methods are available. Two of them depend on *in vivo* tests, the third on an *in vitro* titration.

The first is Ehrlich's classical method of injecting mixtures of toxin and antitoxin subcutaneously into guinea-pigs; and this method is still the official one in many countries (see Report 1923, Prausnitz 1929). It has been found convenient to employ the end-point of toxæmic death rather than the end-point of complete neutralization—the L— rather than the Lo dose of toxin (see p. 239). The first procedure is, then, to determine the smallest amount of a suitable toxic filtrate that, when mixed with one unit of standard antitoxin and injected into a 250 gm. guinea-pig, will, on the average, kill the animal by the 4th day. In practice batches of guinea-pigs are injected with each mixture prepared and the mixture producing a 50 per cent. mortality, or thereabouts, is regarded as containing the L+ dose of toxin. The procedure is then reversed. The amount of toxin is held constant at the L+ dose, and the amount of the serum to be tested is varied, again using an adequate number of guinea-pigs for each dose in the range within which the L+ mixture is expected to fall. The amount of serum in the mixture that gives a 50 per cent. mortality within 4 days contains one unit of antitoxin. The number of guinea-pigs that must be inoculated with any such mixture in order to give an estimate of the unit value of an antitoxic serum within a specified margin of error depends on the factors considered in Chapter 43. In practice a rough preliminary titration is made, and the exact value of the serum is then assessed using larger numbers of guinea-pigs.

The second method depends upon the observation of Römer (1909) that the intradermal injection into a guinea-pig of 1/250–1/500 M.L.D. of toxin is followed by a localized swelling and erythema. With slightly larger doses, this erythematous reaction is followed by definite necrosis. The neutralization of toxin by antitoxin prevents this reaction.

The great advantage of this method is that it allows the toxicity of several different mixtures to be tested on a single guinea-pig, and thus not only economizes animals but eliminates much of the difficulty due to differences in susceptibility between one guinea-pig and another. The successive steps in the process of standardization do not differ in any essential from those followed in the Ehrlich method. Varying amounts of a suitable toxin are first mixed with one unit of the standard antitoxin and 0·2 ml. of each mixture is injected into the depilated skin of a guinea-pig. The amount of toxin in the mixture that gives a minimal skin reaction is noted. This amount of toxin has been defined by Glenny and Allen (1921) as the Lr dose. A series of mixtures is now prepared in which the Lr dose of toxin is mixed with varying amounts of the serum under test, and 0·2 ml. of each of these mixtures is injected intradermally into another guinea-pig. The amount of serum in that mixture that gives a minimal skin reaction contains one unit of antitoxin. In practice it is customary to use fractions of 1.A.U. and corresponding fractions of the Lr dose of toxin, in order to avoid the administration of lethal doses of toxin in the mixtures containing toxin in excess.

The third method depends on the observation of Ramon (1922) that a satisfactory measure of the combining power of an antitoxic serum can be obtained by mixing falling amounts of the serum with a constant amount of toxin and noting the ratio of one reagent to the other in the tube that first shows flocculation. This is the method of optimal proportions (see p. 202). Glenny and Okell (1924) (see also Glenny and Wallace 1925) have suggested that the amount of toxin corresponding to one unit of antitoxin in the mixture that shows optimal flocculation when tested by the Ramon method should be called the Lf dose. The procedure with this *in vitro* test is essentially the same as with either of the *in vivo* methods. The Lf dose of a suitable toxic filtrate is determined by titration against the standard antitoxin. The amount of the serum under test that gives optimal flocculation with the Lf dose of toxin is determined by a second titration. This amount of serum contains one unit of antitoxin.

Table 114 (see Glenny 1925) sets out the units (or named doses) employed in testing diphtheria toxin and antitoxin. The meaning to be attached to the Schick dose has been considered on p. 1377.

TABLE 114
(After Glenny 1925.)

Character measured.	Unit or Dose.		Result of Inoculation in Guinea-pig.
	Toxin.	Antitoxin.	
Toxicity . . .	M.L.D.	—	Death on 4th day.
„ . . .	M.R.D.	—	Minimal skin reaction.
Combining power	L+ dose	1 A.U.	Mixture causes death on 4th day.
„ „	Lo „	1 A.U.	Mixture causes minimal œdema.
„ „	Lr „	1 A.U.	Mixture causes minimal skin reaction.
„ „	Lf „	1 A.U.	Mixture gives optimal proportions for flocculation.
„ „	Schick „	0·001 A.U.	

The comparison of large numbers of different antitoxic sera—natural or concentrated—by these different methods has brought to light facts of considerable theoretical and practical importance. The L+ dose of toxin is always appreciably larger than the Lo dose. The Lr dose of toxin always approximates closely to the Lo dose, as would be expected, since a very small excess of unneutralized toxin will elicit the Römer reaction. The Lf dose is, in general, slightly less than the Lr dose. Glenny (1925) notes the following relation between the various doses of an average toxic filtrate: L+ dose = 0·21 ml., Lo dose = 0·18 ml., Lr dose = 0·175 ml., Lf dose = 0·155 ml. The ratio of the Lf

dose to any of the *in vivo* doses is not constant for all toxic filtrates. Antitoxin gives flocculation with both toxin and toxoid while the determination of the L+, Lo and Lr doses depends on the presence of unneutralized toxin, and is hence affected by differences in the proportion of toxin to toxoid. Moreover, as was shown by Glenny, Pope and Waddington (1925), not only does the Lf/Lr ratio vary from one toxic filtrate to another, when these are tested against the same serum, but the Lf/Lr ratio of a single toxic filtrate varies when it is tested against different antitoxic sera. It follows that, when sera are compared with one another by *in vivo* and *in vitro* methods, their apparent relative potency may vary according to the method of comparison employed: and, with such sera, the ratio $\frac{\textit{in vitro}\text{ value}}{\textit{in vivo}\text{ value}}$ will vary from one serum to another. Glenny and his colleagues note that they have obtained *in vitro*/*in vivo* ratios varying from 0·4 to 2·0 with different antitoxic sera, and that, in general, if the Ehrlich value is considerably higher than the Ramon value *i.e.* if the *in vitro*/*in vivo* ratio is low, the serum will be found to give rapid flocculation; while, if the Ramon value is higher than the Ehrlich value, the serum will be found to give very slow flocculation and the toxin-antitoxin complex will show considerable dissociation on simple dilution. In their later papers, Glenny and his colleagues use the inverse ratio—*in vivo*/*in vitro*—so that a serum with a ratio greater than unity has a greater protective action than its flocculation value would lead one to expect.

Such findings as these clearly raise the question as to whether the protective action of an antitoxic serum is determined solely by its antitoxic content, as measured by one or other of the methods commonly employed. This question has been discussed on p. 1030, where it was noted that antitoxic sera may differ in *avidity*, as well as in their total capacity for neutralizing toxin. It was noted also that this avidity appears to be determined by the firmness of the toxin-antitoxin union, and that avid sera (those that unite firmly with toxin) are more effective than non-avid sera, those that form a loose union with toxin.

The avidity of antitoxic sera appears to be determined, at least in part, by the type of serum protein with which the antitoxin is associated. Thus (Barr and Glenny 1931*a*, *b*, Glenny and Barr 1932, Glenny *et al.* 1932) if successive globulin fractions are precipitated with increasing amounts of ammonium sulphate, the earlier fractions show a higher *in vivo*/*in vitro* ratio, a higher dilution ratio and a greater curative power in rabbits than the original serum, while the avidity as judged by these tests decreases with each successive fraction precipitated.

Non-avid sera do not form a high proportion of the antidiphtheria sera obtained in routine serum production, and Glenny and his colleagues note that they are usually derived from horses that have proved difficult to immunize. With other antitoxic sera (see Glenny *et al.* 1932) it would seem that they may occur more frequently.

Interesting light has been thrown on these findings by the more recent observations of Kekwick and his colleagues (Kekwick and Record 1940, Kekwick *et al.* 1941). They find that, of the three globulin fractions separable by the electrophoretic method from diphtheria antitoxic horse serum, the α component has no detectable antitoxic activity. The antitoxic β and γ components, which together account for the total activity of the serum, differ from each other in several respects, notably in their flocculation time, the composition of their floccules, and their *in vivo*/*in vitro* ratio. The γ component flocculates more rapidly in the presence of toxin, and has therefore a higher *in vivo*/*in vitro* ratio; the floccules formed contain twice as much antitoxin nitrogen as those formed by the β globulin. In the horse's serum the γ component appears earlier during the course of immunization than the β, which, however, continues to increase as further injections of toxoid are given. The γ antitoxin precipitates at lower salt concentrations than the β antitoxin, and tends therefore, during the ordinary concentration process, to be salted out with the euglobulin fraction and discarded, so that the bulk of the antitoxin remaining in the pseudoglobulin fraction is of the β type. The proteolytic method used in the preparation of refined serum also favours the retention of the β at the expense of the γ antitoxin.

It is apparent that the proportion of the β and γ components in different batches of therapeutic serum is subject to variation. The suggestion is that the avidity of the serum is determined to some extent by the proportion of γ globulin, and that batches of sera high in this respect are likely to be of greater therapeutic efficacy than others containing mainly β globulin. Such an explanation is not inconsistent with McSweeney's (1941) observations on the successful results obtained with a particular serum in the treatment of cases of hypertoxic diphtheria (see also Trevan 1941).

These findings do not, of course, lessen the importance of determining the antitoxin content of a serum in terms of standard units, or of producing sera with the highest possible antitoxin content. We clearly want to know how many units we are giving, and to give as many units as we can. They do suggest that the protective or curative value of a serum is not always completely described by stating the number of units it contains; and it may prove desirable in the future to adopt some standard of avidity in addition to our standard of content.

The Treatment of Carriers.

Thomson, Mann, and Marriner (1928–29) found that a high proportion of pure throat carriers became negative after tonsillectomy. Valuable as this method is, it is limited in its application to throat carriers, who constitute only about 10 per cent. of the total. Up till recently little could be done to free the nasal carrier, but the introduction by Delafield, Straker and Topley (1941) of sulphonamide snuff has revolutionized the treatment of these patients. The inhalation of 33 per cent. sulphathiazole in 67 per cent. magnesium carbonate about 6 times a day cures the great majority of nasal carriers within a fortnight. If an associated hæmolytic streptococcal infection is present it may be advisable to use pure sulphanilamide snuff, as recommended by Boissard and Fry (1942).

The Epidemiology of Diphtheria.

Before discussing the prophylaxis of diphtheria it is necessary to have in our minds a picture of how the disease behaves under natural conditions. This picture, as it has been presented to us by the combined studies of bacteriologists and epidemiologists, using the Schick test and the throat swab, is far more detailed and informative than the corresponding picture that we possess for other diseases. For this reason it was selected as an illustrative example in our general discussion of herd infection and herd immunity in Chapter 56, and reference should be made to that chapter. We may add here a few additional particulars with regard to the natural history of the disease, that were not relevant to the more general discussion.

Diphtheria is a disease which shows a characteristic age incidence. Greenwood (see Andrewes *et al.* 1923) has calculated the probability of being attacked by diphtheria at different ages for three samples of the population, in different places and at different times. The figures selected were those for Breslau (1886–90), Manchester (1911–12), and London (1910–12). In the case of London the rates for males and females were calculated separately. In Breslau the peak of the curve falls in the age group 2–3, with a slight decline in the group 3–4. In Manchester the maximum is at 3–4, with a slight decline at 4–5. In London the maximum for both sexes is at ages 4–5. All four curves are definitely skew, the mode falling well to the left. Diphtheria is rare during the first year of life, and then becomes increasingly frequent until the maximum frequency is reached somewhere between the 2nd and 5th year. The incidence declines slowly until the age group 5–10, then rapidly during the age group 10–15, after which the probability of contracting diphtheria is small. It becomes still smaller in the age

group 15–20, and remains at a very low level during the rest of life. These figures, it must be realized, refer to the behaviour of diphtheria before the introduction of artificial immunization. The distribution of clinical cases of disease in a population a considerable proportion of which has received prophylactic inoculation is usually very different, and is determined largely by the age and sex of the unprotected members of the community.

Apart from this variation in susceptibility at different ages, diphtheria shows a curious capriciousness in its incidence among small communities containing many members of susceptible age. The attack rate among those exposed to risk may be very low, and varies very widely from one outbreak to another.

Diphtheria is not markedly associated with density of population, if we measure that elusive variable by computing the total population within some unit area; indeed, over considerable periods in its history, the disease has shown a marked predilection for rural as opposed to urban conditions. It is, however, typically a disease of schools, and of institutions where children are herded together at susceptible ages. Moreover, a particular school or institution may form an endemic centre of the disease for a period of months or years.

Under conditions in which the majority of cases of clinical diphtheria are removed to an isolation hospital, it is probable that the spread of the disease is due mainly to the occurrence of carriers (see Savage 1909, Doull and Lara 1925). We have previously given figures for the frequency of carriers of *C. diphtheriæ* among various classes of contact, and among the population at large. The carrier state is by no means confined to those who have themselves passed through an attack of disease; but, since the diagnosis of clinical diphtheria at once brings a patient under observation, and involves some degree of control and a considerable degree of responsibility on the part of the medical authorities, it is of interest to note the rate at which the proportion of carriers decreases during and after convalescence.

An investigation carried out by Hartley and Martin (1919–20) has yielded valuable information on this point. The study was made on 457 young adults suffering from faucial diphtheria. A swab was taken on the day of admission, and every 7th day thereafter, till three successive negative results were obtained. The time at which any individual ceased to carry *C. diphtheriæ* was calculated as being half-way between the last positive and the first of three successive negative swabs. The data were complete for the first 50 days, after which some of the patients were no longer under observation. The results were plotted in the ordinary way, using either the numbers still carrying, or the logarithms of those numbers, as ordinates, and time as abscissæ. It was found that the latter curve after the first five days from admission, gave a close approximation to a straight line. The general form of the equation of such a curve is $\frac{\log n_1 - \log n_2}{t_2 - t_1} = \mathrm{K}$, where n_1 and n_2 are the numbers carrying at times t_1 and t_2. If we take time as measured from the fifth day after admission, this may be expressed in the form

$$\log n = \log n' - \mathrm{K}t,$$

where n is the number carrying on any given day, n' is the number carrying on the 5th day, and t is the time interval in days measured from the fifth. The equation to the curve which gave the best fit was calculated by Greenwood as

$$\log n = 2{\cdot}6002 - 0{\cdot}0218\, t.$$

The observed and calculated number of carriers for the first 50 days were as given in Table 115.

TABLE 115

Days after Admission.	Number carrying (observed).	Number carrying (calculated).
5	392	—
10	302	310
15	232	242
20	194	189
25	156	147
30	118	115
35	92	89
40	70	70
45	52	54
50	41	42

The agreement is obviously very close, and such a relationship between lapse of time and the disappearance of bacilli suggests that this disappearance is due to the operation of a large number of small causes acting independently, in other words to chance. It would seem that a person who has carried *C. diphtheriæ* for 6 weeks or more is, on the average, just as likely to become free during the next few days as another who has carried for only a week. There are, however, as Hartley and Martin point out, a few persons with obviously unhealthy tonsils who carry persistently, but cease to carry after tonsillectomy.

It may be noted that the constant 0·0218 in the formula quoted above indicates that about 5 per cent. of those carrying on any one day have ceased to carry on the day following. In order to determine whether this rate of clearing held true in general, or applied only to this particular sample, data collected from various published reports were analysed in the same way. It was found that the general form of the curve was very similar in each case, *i.e.* the logarithmic relation between carrier rate and time held good, but the value of the constant varied very widely, such figures as 0·018, 0·093, and 0·202 being obtained. Some adjustment is necessary because, in certain of these series, the test of clearance was a single negative swab, but the value of the constant in Hartley and Martin's series would only be altered to 0·032 on this basis, so that the rate of clearing under different conditions varies considerably, a fact which seems to merit further investigation.

A larger and equally illuminating series of figures recording the apparent rate of disappearance of diphtheria bacilli in convalescents at the North-Eastern Hospital has been published by Thomson, Mann and Marriner (1928–29). Nose and throat swabs from proved cases of diphtheria were taken once during the 2nd and once during the 3rd week, and after that twice a week till six consecutive negative cultures had been obtained. Cases in which the bacilli persisted for over 12 weeks from the beginning of the illness were regarded as carriers. Of 3,208 cases investigated, 242, or 7·5 per cent., became carriers. Table 116 shows the proportion of cases, exclusive of carriers, that became negative in successive weeks.

It will be seen that infection disappeared from the throat much quicker than from the nose. Analysis of these figures showed that when the throat only was affected the rate of clearance was practically the same at all ages, but that when the nose or nose and throat were affected the rate of clearance was slower in younger than in older patients. Of 285 carriers on which information is afforded, including the 242 already mentioned, 255, or 89·5 per cent., were infected in the nose or nose and throat, 26 in the throat only, and 4 in the ear only. The average time spent in hospital was roughly 7 months, and the longest 21 months. The age distribution of the total cases is not given, but it is mentioned that 55·7 per cent. of the carriers were under 7 years of age and only 4·5 per cent. over 13 years of age.

TABLE 116.

PROPORTION OF CONVALESCENTS BECOMING NEGATIVE EACH WEEK.

	Throat.		Nose, or Nose and Throat.	
	No.	%	No.	%
Within 2nd week	861	49·9	298	24·0
„ 3rd „	255	14·8	159	12·8
„ 4th „	254	14·7	184	14·8
„ 5th „	138	8·0	158	12·7
„ 6th „	86	5·0	136	11·0
„ 7th „	46	2·7	97	7·8
„ 8th „	41	2·4	52	4·2
„ 9th „	18	1·0	51	4·1
„ 10th „	15	0·9	45	3·6
„ 11th „	5	0·3	32	2·6
„ 12th „	7	0·4	28	2·3
Total	1,726	100·1	1,240	99·9

Wright (1941*a*), who records his experience at Liverpool on the rate of disappearance of diphtheria bacilli from the nose or throat of 311 convalescent patients, obtained results similar to those recorded by Hartley and Martin (1919–20). He made the interesting observation that clearance was most rapid in patients infected with the *intermedius*, and slowest in those infected with the *gravis* type. Examining swabs taken at weekly intervals and inoculated on to tellurite blood agar slopes, he found that a negative result was often followed by a positive, and that in 2·5 per cent. of patients three successive negatives were followed by a positive. The customary demand for three consecutive negative results does not therefore ensure freedom of the patient from infection on discharge. The unfavourable effect of an associated hæmolytic streptococcal infection on the persistence of diphtheria bacilli, especially in the nose, has been commented on by Boissard and Fry (1941, 1942).

We have already noted the effect of closeness of contact on the carrier rate. It may be added that the investigation of epidemics in institutions suggests that this factor is of great importance, both in regard to the production of carriers and of actual cases of disease. It would seem that duration of contact must be considered as one aspect of this problem; and there is good evidence that contact of the type which is exemplified by the case of boys sleeping in a single dormitory is enormously more important than casual or momentary contact during working hours (Dudley 1923).

In concluding this section we may consider the importance of *fomites* in the spread of diphtheria. From the case of the child's box of bricks, reported by Abel in 1893, there have been instances in which it seemed possible to incriminate a particular article as a disseminator of infection. Very occasionally, as in the case of the common supply of penholders for a number of schoolboys recorded by Dudley (1923), it would appear that such a mode of transmission may have played a significant part. In general, however, the balance of evidence would appear to be heavily against the view that fomites play any significant rôle in the spread of the disease; and there is nothing to suggest that stringency of disinfection would make any appreciable impression on its incidence. An exception must, however, be made for *dust*, which observations by Wright and his colleagues at Liverpool (Wright, Shone, and Tucker 1941, Crosbie and Wright 1941) have shown

to be a grave source of danger in hospitals and other institutions. Crosbie and Wright were able to isolate diphtheria bacilli in large numbers from floor dust in the neighbourhood of diphtheria patients, and to show that they might remain fully virulent for as long as five weeks. They are of the opinion that floor dust distributed by sweeping and blanket dust liberated during the making of beds may play an even more important part in cross-infection in diphtheria wards than the direct transmission of droplets from patient to patient. Measures to reduce aerial infection by dust, such as the application of heavy oil to the floors (van den Ende, Lush and Edward 1940, Thomas 1941) and to the blankets (van-den Ende and Thomas 1941) hold out some promise of success, though so far no simple method for destroying diphtheria bacilli in the dust itself has yet been devised.

The Spread of Diphtheria by Domestic Animals.—It was at one time believed that certain domestic animals played a significant part in the spread of diphtheria, but most of the evidence on which this belief was based has been shown to be erroneous. It is now clear that Klein's (1889) statements with regard to diphtheria in the *cat* were incorrect, and Savage (1919–20) has shown that this animal is extremely resistant to infection by feeding, by intranasal injection, or by swabbing the throat with living culture. There is, indeed, little evidence that it ever contracts or transmits the disease, though one or two suggestive cases have been recorded by Simmons (1920). The domestic *fowl* was credited with similar malign potentialities, a belief that probably arose as the result of a superficial similarity between "fowl diphtheria" and the human disease. The fowl disease is now known to be caused by a filtrable virus, and the generally accepted view is that fowls can be disregarded as a potential source of infection. A report by Litterer (1925) does, however, suggest that these birds may sometimes harbour virulent *C. diphtheriæ*. In two instances he isolated diphtheria bacilli from the throat of several fowls, kept by families among which a case of diphtheria had occurred. The bacilli were fully virulent as tested by the inoculation of guinea-pigs, controlled by protection with diphtheria antitoxin. Some of these fowls were obviously ill, and showed typical membranes. They recovered rapidly after injection with antitoxin. Attempts to reproduce the disease in healthy fowls by swabbing the throat with virulent *C. diphtheriæ*, with or without previous scarification, were uniformly unsuccessful. Thirty fowls suffering from roup were treated in a similar manner. In five cases a typical membrane was produced. If similar results are recorded by other investigators it may be necessary to consider the possibility of the fowl playing some part in the spread of the disease in exceptional circumstances; but it seems altogether unlikely that this part is a significant one from the epidemiological point of view.

We have referred (see Chapter 49) to the occasional isolation of virulent diphtheria bacilli from the *horse*, but here again there is no evidence that such infection has any appreciable effect on the incidence of the disease in man.

Similarly, the demonstration by Ramon and Erber (1934) and by Dold and Weigmann (1934) of virulent diphtheria bacilli in the throats of *monkeys* has little significance in relation to human infection under ordinary conditions of life.

The *cow* is in a different category, since the general consumption of milk renders any infection of this animal of particular importance. The earlier records of diphtheria in the cow may be safely disregarded, since the nature of the disease was never fully established, and the conditions described were, in most instances,

subsequently shown to be due to quite different causes. It is now reasonably certain that the cow does not naturally suffer from any form of infection with *C. diphtheriæ*, and that the only lesion in this animal which has any significance in the spread of the disease is the occasional infection of pre-existing superficial lesions on the teats from the hands of a milker who is suffering from the disease, or is carrying virulent bacilli. The existence of these lesions has been recorded by Dean and Todd (1902), Ashby (1906) and Henry (1920), and the authors have met with one instance of this condition. In all cases the virulence of the bacilli has been established. Such infected wounds, when they occur, are of obvious importance as forming a persistent focus from which the milk is likely to be infected; and in the cases recorded the lesions have been detected during the investigation of a milk-borne epidemic. In the majority of the cases in which an epidemic of diphtheria is traceable to an infected milk supply it is probable that the original source of the bacilli has been a human carrier, who has handled the milk at some time during its preparation.

It may be added that the incrimination of the milk rests, in almost all instances, on epidemiological evidence. The isolation of *C. diphtheriæ* from milk, even when a particular supply is under grave suspicion, has in the past been very uncommon; nevertheless a few successful results have been reported (Bowhill 1899, Eyre 1899, Marshall 1907, McSweeney and Morgan 1928). With the introduction of tellurite medium the technical difficulties of isolation are greatly reduced, as is shown by the reports of Fry (1941) and Goldie and Maddock (1943). There remains, however, the difficulty that the actual milk which conveyed the infection is seldom, if ever, available for examination at the time when attention has been drawn to it, so that unless the contamination continues for some days an infected sample is unlikely to be forthcoming. It must be emphasized that the identification of a diphtheria-like bacillus isolated from milk requires the most stringent tests. No organism derived from such a source should be reported as *C. diphtheriæ* until its character has been established by a properly controlled virulence test.

Prophylaxis in Diphtheria.

Our present methods of controlling diphtheria as a herd disease depend on the isolation of patients, the segregation of recognized carriers, and prophylactic immunization. The last method comprises both active immunization by means of an antigenic preparation now usually referred to as diphtheria prophylactic, and passive immunization conferred by the injection of antitoxic serum. Passive immunization, because of its transitory nature, has a very restricted application and will be dealt with later (p. 1401). For all ordinary field purposes active immunization is employed. An adequate understanding of the principles that govern the application of this method to the control of the disease is so important to the medical practitioner and the medical officer of health that the relevant data must be set out in some detail. We shall begin by describing the various forms of diphtheria prophylactic and their standardization, proceed to a consideration of their application under varying conditions in practice, and then discuss some of the results that have followed their use in mass immunization.

The Various Forms of Diphtheria Prophylactic.

As early as 1892 von Behring and Wernicke showed that susceptible animals might be safely immunized by inoculating them with increasing doses of living

culture after a protective dose of antitoxic serum. Six years later Nikanaroff reported successful results from the inoculation of a few doses of toxin neutralized with antitoxin; and Dreyer, in 1900, proposed the use of toxin-antitoxin mixtures during the early stages of antitoxin production in horses. For various reasons there was a long lag before similar methods were applied to man; and it was not till 1913, when von Behring reported the successful use of a toxin-antitoxin mixture for the immunization of children, that inoculation against diphtheria entered the field of prophylactic medicine. From then onwards the use of such mixtures for the active immunization of children developed very rapidly, particularly in the United States under the influence of Park and Zingher (Park 1913, 1918, 1922, Park and Zingher 1915, 1916, Zingher 1921, 1922). Toxin-antitoxin mixtures were found to be not without danger (see Park and Schroder 1932), and after the discovery by Glenny and Südmersen (1921), Glenny and Hopkins (1923), and Ramon (1923, 1928) that diphtheria toxin could be so altered by treatment with formalin as to deprive it of its toxicity without destroying its antigenic power, formol toxoid, or anatoxin as it is called in French-speaking countries, gradually began to replace the older mixtures. The further demonstration by Glenny (1930) that formol toxoid could be improved by precipitation with alum has now placed at our disposal an agent of high antigenic potency which, when properly prepared and used, can be relied upon to give a fairly high degree of protection against diphtheria.

Toxin-Antitoxin Mixture.—In preparations of this class sufficient antitoxin is added to neutralize the toxic effects of the toxin without completely destroying its antigenic activity. The toxin, that is to say, is under-neutralized. Though very satisfactory results have been obtained by the use of such mixtures, a few accidents have shown that the union of toxin and antitoxin is unstable and is liable to be disturbed by unsuitable methods of preparation or of storage. In one instance, in which 5 deaths and 40 serious reactions followed the use of this prophylactic (see Forbes 1927), it was found that, in forgetfulness of the Danysz phenomenon, the toxin had been added in fractions to obtain the correct toxin-antitoxin ratio. In another instance a particular phial of toxin-antitoxin mixture that had been frozen was found to have caused severe constitutional reactions, whereas other phials of the same batch that had not been frozen had been used without any untoward results. Further investigations revealed that the freezing of such mixtures frequently resulted in a considerable rise in toxicity. Pope (1927) has shown that this phenomenon is dependent, in part at least, on a separation of the phenol during freezing; this leads to a local concentration greatly in excess of the 0·5 per cent. or so added for preservative purposes, and to a consequent destruction of antitoxin, which is more sensitive than toxin to phenolization. Observations recorded by Robinson and White (1928) indicate that, even in the absence of added phenol, dissociation of toxin-antitoxin mixtures may occur on freezing, the degree of dissociation depending apparently on the concentration of the mixture. A dose of toxin equivalent to 12 M.L.D. for a guinea-pig may be fatal to a child (Schmidt 1942).

Formol Toxoid. F. T.—This is prepared by treatment of culture filtrates with formalin in a concentration depending on the amount of amino-nitrogen present. The pH of the toxin is adjusted to 7·4–7·6, and the formolized mixture is incubated for 3–4 weeks at 37–38° C. until the product, as judged by the intracutaneous inoculation of guinea-pigs and rabbits, is devoid of all residual toxicity (Barr, Pope, Glenny and Linggood 1941). The chemical changes involved in the conversion of toxin into toxoid are still imperfectly understood, but reference may be made to the observations on this subject of Kissin and Bronstein (1928, 1930), Hewitt (1930), Bunney (1931), and Schmidt (1933*a*, *b*, *c*).

Toxoid-Antitoxin Mixture.—The addition of antitoxin to formol toxoid is an empirical attempt to obtain a reagent that induces an effective immunity without causing a troublesome local reaction, as formol toxoid alone may do, and without running the risk of danger following possible dissociation, such as attends the use of a toxin-antitoxin mixture. It has been developed mainly in Great Britain; in the United States its advantage over simple formol toxoid has always seemed questionable. It is frequently designated T.A.M., but since this abbreviation is also employed by some workers for toxin-antitoxin mixture, it is better avoided unless the particular prophylactic to which it is intended to refer is specifically mentioned.

Toxoid-Antitoxin Floccules. T.A.F.—This is a suspension of the precipitate of floccules that are formed when toxoid and antitoxin are mixed in appropriate neutralizing amounts. The elimination of much of the non-specific serum protein and bacillary products present in toxoid-antitoxin mixtures results in a product that combines effectiveness with a minimum of unpleasant local effects (see Glenny and Pope 1927, Swyer 1931, Dudley 1932, Underwood 1934, and others).

Alum-Precipitated Toxoid. A.P.T.—The use of alum-precipitated toxoid for active immunization in man followed the demonstration by Glenny (1930) that this reagent was extremely effective in inducing immunity in experimental animals (see also Glenny and Barr 1931, Glenny *et al.* 1931). Its high potency appears to be due to delayed absorption of the precipitated toxoid from the site of inoculation, with a consequent prolongation of the antigenic stimulus. It consists essentially of a suspension of the washed precipitate produced by the addition of alum to formol toxoid. Considerable care has to be exercised over its preparation, and exposure of the toxoid to acid or to phenol, such as is inherent in the method described by Watson, Taggart, and Shaw (1941), must be avoided (Glenny *et al.* 1926, Hartley 1935*a*, Barr *et al.* 1941). Fortunately Glenny and his colleagues (Barr, Pope, Glenny and Linggood 1941) have provided a detailed description of how A.P.T. should be made, and the resulting product has been proved by field trials to be both effective and reliable (see also Pope and Linggood 1939, and Seal and Johnson 1941 on purification of toxoid and A.P.T.).

Active Immunization.

To produce a high degree of immunity against diphtheria by prophylactic inoculation, account must be taken of the nature of the prophylactic, the dosage, and the interval between the doses. Other factors, such as the latent immunizability of the population (Dudley, May, and O'Flynn 1934), and possibly the volume of the fluid in which the prophylactic agent is injected (Hartley 1935*b*), may influence the result, but need not be considered here.

Of chief importance is the *antigenic quality of the prophylactic*. As was pointed out in the last section, methods of standardization are still imperfect, and unless a high standard is set, batches of prophylactic may be issued that are unable to stimulate a satisfactory immune response. Even an increase in dosage as much as fivefold may fail to atone for a qualitatively poor antigen (Fulton *et al.* 1942). To Schick-positive subjects who do not react to heated toxin the same *dosage* may be given irrespective of age. In Schick-negative subjects who are being given a single re-inforcing dose, and in Schick-positive subjects who give a pseudo reaction or a Moloney reaction (see p. 1396), it is usually wise to inject a smaller dose in older children and adults than in young children. Toxin-antitoxin and toxoid-antitoxin mixtures, toxoid-antitoxin floccules, and formol toxoid are best administered in three doses at 3-week intervals, the first two in doses of 0·5, 1·0 and 1·0 ml., T.A.F. in 1 ml. doses, and F.T. in doses of 0·2–0·5 ml., 0·5 and 1·0 ml. Alum-precipitated toxoid can be safely relied upon to produce a corresponding,

or even higher, degree of immunity in two doses of 0·2 and 0·5 ml. at 4-weeks' interval. In the early days it was often used in a single dose of 0·5–1·0 ml., but experience showed the results to be unsatisfactory, and the method is now seldom recommended except in special circumstances (see Report 1940).

The choice of prophylactic and the dosage are determined partly by their immunizing power and partly by the local or constitutional reaction to which they give rise. Though exact comparative data are lacking, there is reason to believe that toxoid-antitoxin mixture and toxoid-antitoxin floccules cause the least tissue reaction and that alum-precipitated toxoid causes the most. In general, therefore, it is wise to use the former for Schick-negative subjects, for pseudo-reactors, and for adults whose Schick-reaction is unknown, and to use formol toxoid, or preferably A.P.T., for the rest.

The *interval between the doses* has been shown by numerous workers to play a decisive part in determining the degree of antibody response (for references see Lewis 1941). In a Schick-positive subject who has had no previous experience of the diphtheria bacillus, the first dose of prophylactic has the effect merely of sensitizing the antibody-forming apparatus. The second dose, provided time has been allowed for adequate sensitization to take place, is followed rapidly by the appearance of antitoxin in the circulating blood and the conversion of the Schick-positive to the Schick-negative state. Sensitization is a slow process and till it is complete the full effect of the second dose of antigen will not be experienced; once, however, it is established, it is relatively immaterial how long the second dose is delayed.

Several careful studies have been made on the efficacy of different types of diphtheria prophylactic, as judged by the Schick-conversion rate or by the amount of antitoxin appearing in the blood serum (see, for example, Park and Zingher 1915, 1916, Park 1922, Ramon 1926, Kundratitz 1927, Parish and Okell 1928, Martin, Loiseau, and Laffaille 1928, Ramon and Debré 1931, Seligmann 1931, McGinnes, Stebbins, and Hart 1934, Haine 1935, Fraser and Halpern 1935, 1937, Jensen 1937, Faragó 1940, and for references to results of two doses of A.P.T Pöch and Schmid 1938, Chesney 1939, Fulton *et al.* 1941, Lewis 1941, Volk and Bunney 1939, 1942*a*, Freeman 1942). The findings may be summarized by saying that two properly spaced doses of A.P.T. result in an average Schick-conversion rate of about 98 per cent., whereas with other prophylactics this high rate may be closely approached, but is seldom equalled.

The *duration of immunity* is dependent not only upon the type of prophylactic, but upon the subsequent exposure of the inoculated subject to infection with the diphtheria bacillus. In a community in which there is much diphtheria or a high carrier rate the immunizing mechanism is likely to receive more or less frequent stimuli, which will have the effect of re-inforcing the immunity conferred by the prophylactic. On the other hand, in communities with little or no experience of diphtheritic infection, the antitoxin content of inoculated subjects may be expected to fall progressively (see Jensen 1933*a*, *b*) and the Schick reaction to revert to positive. It is not surprising, therefore, that the results recorded by different workers are not in complete accord. Some observers, for example, like Park (1922), working in densely populated cities, have had few Schick relapses over a period of many years, whereas others, working in areas where there has been little diphtheria infection, have had high relapse rates. Thus Fraser and Brandon (1936), in Canada, found that of children who had received three doses of formol toxoid five years previously, and who had not been exposed to infection

since, 34 per cent. had reverted to the Schick-positive state. Similar results are recorded by Schwartz and Janney (1938) and Volk and Bunney (1942*b*) in the United States, and by Sigurjónsson (1939) in Iceland (see also Parish and Okell 1928, Cooke 1936, Jones 1936-37, Christie 1940, Nevius and McGrath 1940, Duke and Stott 1943). Generally speaking, we may say that in children properly immunized with two doses of A.P.T. and not exposed to diphtheritic infection the Schick-relapse rate should not exceed 5 per cent. per year. Since immunity cannot be relied upon to last indefinitely, it is wise to reinoculate children every 4 or 5 years. For this purpose a single dose of prophylactic is sufficient, since there is abundant evidence to show that a single re-inoculation of persons, even of those who have relapsed to the Schick-positive state, is followed by a rapid rise in the antitoxin content of the blood (see Parish and Okell 1928, Parish and Wright 1938, Fraser 1940, Volk and Bunney 1942*b*, Wishart *et al.* 1944). In this connection it may be noted that many patients who have recovered from diphtheria remain Schick-positive (see Warin 1940). Since second attacks of the disease are not uncommon, such persons may be regarded as susceptible, and inoculated or re-inoculated with the rest. Why an attack of diphtheria is not always followed by immunity is still doubtful. That it is not due mainly to suppression of the antibody-forming mechanism by the administration of large quantities of antitoxin therapeutically seems clear from Madsen's (1939) observation that 13 out of 26 untreated patients failed to form detectable antitoxin as the result of their attack.

The desirability of a *preliminary Schick test* to find out whether inoculation is required, and of a subsequent Schick test to find out whether immunization has been successful, is open to question. Space does not allow a full discussion of the subject, and we must therefore restrict ourselves to a summary of the conclusions we have reached from our own observations and from those of other workers.

In a mass immunization campaign a preliminary Schick test, entailing as it does two inoculations and an extra visit for each child, seems to us to be justified on only two conditions, firstly that a high proportion of the children are Schick-negative, and secondly that inoculation is restricted to Schick-positive reactors. In areas in which diphtheria has not been prevalent and in which inoculation has not been widely practised, a high proportion of the children are generally found to be Schick-positive. Under such conditions Schick-testing is wasteful of both time and material. It is all the more so if, as many experienced workers recommend, the Schick-negative reactors are inoculated at the same time, usually with a single dose, to reinforce their failing immunity. For practical purposes, therefore, we see no advantage in carrying out a preliminary Schick test on a pre-school or school population. The susceptible members require inoculation anyhow, and the immune members will come to no harm through being given two doses of prophylactic but will, on the contrary, have their immunity strengthened.

In adults, the position is different. Mass immunization is seldom called for, so that the extra time required for a preliminary Schick test is a less important consideration. A fairly high proportion will probably be found to be immune; and the argument for inoculating adults who are Schick-negative is not so strong as for children who are still at an age when susceptibility is relatively great. An even more important factor, however, is that the inoculation of adult Schick-negative or pseudo-reactors with full doses of prophylactic, especially A.P.T., is likely to be followed by a severe local and even constitutional reaction. It is

wise, therefore, before inoculating adults, to carry out a preliminary Schick test, and to restrict inoculation to Schick-positive reactors, unless the subject is, like a doctor, nurse, or school teacher, likely to be exposed to an unusually high risk of infection; for such persons a single inoculation with half the usual first dose is recommended. To test the subject's sensitivity to formol toxoid, the Moloney test (Moloney and Fraser 1927) may be carried out either in addition to the Schick test or in place of the heated toxin control. An injection of 0·2 ml. of a 1/20 dilution of formol toxoid is made intradermally. Any person giving more than a minimal local reaction should be immunized very cautiously. In practice, however, this test is seldom needed.

For the mass immunization of children, alum precipitated toxoid, injected in doses of 0·2 and 0·5 ml. at 4-weeks' interval, is the prophylactic of choice. For adults likewise, A.P.T. may be used provided it is confined to Schick-positive reactors (see Leete 1942). Less reaction, however, is likely to be experienced from T.A.F.; this should be given in three doses of 0·5–1·0, 1·0, and 1·0 ml. at intervals of 3 weeks (Circular 1942).

By itself a *post-inoculation Schick test* is of value in learning whether the child has been satisfactorily immunized. It is carried out best 2–4 months after the last inoculation, when the antitoxin content of the blood is at its height. Though such a test may often be usefully applied to persons who are exposed to special risk of infection, it is seldom justifiable in mass immunization campaigns. There is, however, a strong case to be made out for testing a sample of the inoculated population in order to gain assurance that a high proportion, such as 98 per cent., have responded satisfactorily. So many variables enter into the development of Schick-immunity that this should never be taken for granted.

To compare the antigenic potency of two different prophylactics or of different modes of administration of the same prophylactic, it is desirable to estimate the *Schick-conversion rate*. This necessitates preliminary as well as post-inoculation Schick-testing, and is calculated on the proportion of Schick-positive subjects in each group that are rendered Schick-negative. Unless a preliminary test is made, the proportion of original Schick-negative reactors in each group is unknown, and a difference in their distribution will affect the result of the comparison.

The Protective Effect of Active Immunization.

Though a high Schick-negative or Schick-conversion rate affords a strong presumption that satisfactory immunization has been achieved, the ultimate court of reference in determining the efficacy of prophylactic inoculation must be the behaviour of immunized persons when exposed to the risk of natural infection.

Bieber (1920) reported the after-history, from 1913, of 1,097 immunized and 3,275 non-immunized children in villages near Magdeburg. Of the former, 52 (4·8 per cent.) had contracted diphtheria; of the latter, 493 (15·1 per cent.). Park (1922) records the history over a shorter period of 90,000 immunized or naturally immune children and 90,000 controls. During the period of observation 14 of the former contracted diphtheria as against 56 of the latter. Adams (see Fitz-Gerald 1928) records the history during the years 1926 and 1927 of 11,000 children who had received two immunizing doses of toxoid, and of 9,000 non-immunized controls. Among the immunized the incidence of diphtheria was 1·55 per 1,000, among the non-immunized it was 11·44 per 1,000. Isabolinski and others (1931) describe the results of immunization in Smolensk. Of 4,185 children immunized with toxoid, 0·14 per cent. contracted diphtheria during the eleven months following immunization; of 19,000 control children in the same town 1·5 per cent. con-

tracted diphtheria during the same period. McKinnon and others (1931) and Fitz-Gerald and others (1932) record the results of immunization in Toronto. Among a group of 36,189 children who had passed through the immunization centres and had been found to be Schick-negative, or had received one or more immunizing doses of toxoid, or had been found to be sensitive to a preliminary injection of toxoid and so received no further treatment, there were 120 cases of diphtheria during the period of observation (December 1926–June 1929). As a control the prevailing rates of diphtheria morbidity at ages among the non-immunized population were applied to each age group of the immunized. This gave 478 as the expected number of cases. Of the 36,189 children, 16,829 had received three doses of toxoid. Among this group 222 cases of diphtheria would have been expected; only 23 occurred. There are many other records of an analogous kind (see Forbes 1932). More recently, in Scotland, there were 2,833 cases of diphtheria and 13 deaths among about 750,000 immunized, and 17,091 cases and 794 deaths among rather less than half the number of non-immunized persons. The risk of contracting diphtheria was about 14 times less in the immunized than in the non-immunized, and the risk of contracting fatal diphtheria was over 100 times less (A. Russell 1943).

Still more demonstrative evidence is obtained if we confine our attention to groups of persons who are exposed to an unusually high risk of contracting diphtheria. Fever hospitals supply an environment in which such a risk occurs, and the history of attempts to immunize the nursing and domestic staff is correspondingly instructive (see, for instance, Benson 1928, Woods 1928, Harries 1930). We may take the figures given by Harries for the Birmingham City Fever Hospital as an illustrative example (Table 117).

TABLE 117

SHOWING THE DIPHTHERIA INCIDENCE AMONG THE NURSING AND DOMESTIC STAFF AT BIRMINGHAM CITY FEVER HOSPITAL. (Modified from Harries.)

Year.	Diphtheria Incidence per cent.		
	Nursing.	Domestic.	Combined.
1919	15·6	9·4	13·5
1920	24·5	16·3	21·8
1921	13·0	11·1	12·4
1922 (*a*)	6·5	6·7	6·5
1923 (*b*)	3·7	8·9	5·2
1924	0·93	11·1	3·9
1925 (*c*)	—	—	0·77
1926	—	—	0·76
1927	—	—	0
1928	—	—	0
1929	—	—	0·6

(*a*) Schick testing of nursing staff commenced.
(*b*) Active immunization of nursing staff commenced.
(*c*) Active immunization of domestic staff commenced.

The significance of such figures cannot be in doubt. The decline of diphtheria among the nurses coincident with the commencement of Schick testing and immunization, the persistence of diphtheria among the domestic staff until similar measures were applied to them, and the subsequent lowering of incidence until, during 3 years, only 1 case of diphtheria occurred among a staff increasing from 130 to 166, indicate clearly that adequate immunization of all susceptibles will

reduce to insignificance the incidence of this disease among a community exposed to particularly severe risks.

Orphan asylums, children's homes and boarding schools, where persons of a susceptible age are congregated together, afford another example of an environment in which the risk of infection is above the average; and the experience in such institutions confirms that obtained in fever hospitals. Nassau (1930), for instance, records the effect of active immunization in a children's home near Berlin; and Dudley (1923, 1926, 1932) has provided a very fully documented account of the control of diphtheria in the Royal Naval School at Greenwich.

There is, then, no reasonable doubt that active immunization, properly performed, is highly effective in protecting the individual against diphtheria, and in eliminating diphtheria from any small closed community in which all the susceptible inmates are immunized. Emphasis, however, must be placed on the word "properly." This implies using a high-quality prophylactic in adequate dosage with a suitable interval between the doses, controlling the effect by Schick-testing a sample of the population subsequently, and giving a re-inforcing dose at 4- or 5-year intervals till the child is 15 years of age. Unless these conditions are complied with, cases of diphtheria and even occasional deaths, may be expected to occur, as the experience of Glover and Wright (1942) at Liverpool shows. It may be noted that in the instances in which infection in an immunized person, or in a fully immunized community, has been recorded, the infection has nearly always been of the *gravis* or *intermedius* type, and the case mortality has been considerably less than in non-immunized persons (see Dudley *et al.* 1934, Parish and Wright 1935, Glover and Wright 1942).

The Results of Mass Immunization.

We may now turn to the question whether prophylactic inoculation, controlled when necessary by Schick-testing, will reduce significantly or eliminate altogether the incidence of diphtheria in the community at large, if applied on the widest possible scale. It must be realized that the emphasis is on the final limiting clause. There is no reason to doubt that, if all susceptibles in the community could be immunized, the reduction in the incidence of diphtheria would be of the same order as that attained in closed or semi-closed communities. But we are dealing with practical politics, and what we want to know is the probable effect of an administratively possible policy. We are here faced with a problem of great statistical difficulty. It will not do to cite a few instances in which the inauguration of mass-immunization of school children has been associated with a decline in the general diphtheria morbidity rate, because we know quite well that most infective diseases are subject to secular trends in frequency, and a falling rate for any one of them might well have no significant association with any particular preventive measure adopted at the time the fall occurred.

Woods (1928), for instance, has pointed out that the fall in the diphtheria morbidity rate which followed the introduction of mass immunization in New York in 1918 cannot be accepted as certainly significant. The rate was already falling before the immunization began; and if a straight line is fitted to the trend of mortality for the years prior to 1917, and is then extrapolated beyond 1918, this extended line approximates very closely to that which would describe the actual course of events during the following decade.

Godfrey (1932), in an admirable review of the available evidence, discusses the possible reasons for such divergent results as those recorded in New York City and Philadelphia, where immunization was followed by a marked decrease in morbidity, and in Newark,

Detroit and Buffalo, where similar measures failed. Tracing in detail the course of events in eleven different districts in New York State he arrives at the conclusion that the crucial point is to be found in the immunization of the child of pre-school age (the age group 1–5). The earlier attempts at mass immunization were, for obvious administrative reasons, confined mainly to children of school age (the age groups 5–9 and 9–14). Godfrey can find no evidence that the immunization of 50 per cent. or over of the children in these age groups produces a definite fall in the incidence of diphtheria in the community as a whole. When, however, the immunization of 30 per cent. or more of the age group 1–5 is superimposed on that of the later age groups there is an immediate and striking decline in the general prevalence of the disease. Godfrey found only two exceptions to this rule in the eleven areas from which his data were obtained.

The same point is made by Lee (1931) who has studied the trend of diphtheria mortality in Philadelphia and in New York City from 1870 to 1930. Fitting an exponential curve to the recorded death rates, he finds that for neither city was there any significant departure from the general trend between the end of the period (1890–1900), when the use of antitoxin as a therapeutic measure was becoming general, and 1929. But in 1930, following the extension of the immunization campaign to children of pre-school age, there was a dramatic change in both cities. In Philadelphia the expected death rate on the basis of the previous trend would have been 10·5 per 100,000, with odds of 20 : 1 that it would fall between 8·1 and 13·6. Actually it was 2·86. In the case of each city the odds against the observed difference between the actual and the expected death rates being due to chance are enormous. (See also Deadman and Elliott 1933.)

In summary, we have in the modern method of antidiphtheria immunization a measure that is based on well-attested immunological principles, involves the use of a reagent of proved potency, is supported by the results of numerous animal experiments, has been shown to be effective in raising the resistance of the individual and in controlling the incidence of the disease in isolated or semi-isolated communities, and is almost certainly capable of greatly reducing the incidence in the community as a whole, if due attention is paid to herd reactions.

It is possible that the effect of immunizing children of school age alone may be offset, so far as its influence on the *general* morbidity is concerned, by the creation of healthy carriers who may infect the younger susceptibles in their homes. We have, here, an admirable example of the necessity for considering the factors that determine herd immunity as well as those that determine immunity in the individual. These factors are very difficult to analyse. The results of a recent survey in the United States and Canada are summarized in Table 118.

Many of the figures given are probably subject to a considerable sampling error; nevertheless the differences between them are often so great as to leave no doubt of their significance. No constant relationship is evident between either the proportion of children immunized, the proportion of Schick-positive reactors, or the carrier rate of virulent diphtheria bacilli to the morbidity from clinical diphtheria. Cleveland, for example, with about the same proportion of susceptibles and the same carrier rate as Virginia, had a morbidity rate 40 times less. Alabama, with only 9 per cent. of susceptible children, had a morbidity rate 12 times as great as Kingston, which had 3 times as many susceptibles. Schuman and Doull (1940) estimate that during 1937–39 in Cleveland there were about 500 carriers of virulent diphtheria bacilli to every case of clinical diphtheria, whereas the corresponding figure given by Frost (1928) for school children in Baltimore in 1921–22 was 47 carriers to each case. Such data as these must make us pause before concluding that immunization alone is sufficient to eradicate diphtheria from a population.

There is here a fascinating problem for investigation, the solution of which is likely to be of both academic interest and administrative importance.

TABLE 118.

RELATION BETWEEN CARRIER RATE AND INCIDENCE OF DIPHTHERIA IN A PARTLY IMMUNIZED POPULATION.

Observations made chiefly during 1937–39.

Observer.	Place.	Approximate Percentage of Children Immunized.	Percentage of Schick-positive Reactors.		Per cent. virulent K.L.B. Carrier Rate in Children.	Morbidity Rate of Clinical Diphtheria per 100,000 Population.
			All Children.	Unimoculated Children.		
Schuman and Doull (1940)	Cleveland, Ohio	80	36	78	0·56	1·67
Stebbins (1940) .	Kingston, N.Y.	77	27	64	0·03	3·5
Fraser and Halpern (1940)	Toronto	? but probably high	?	82[1]	0·04	5·5
Gill (1940) . . .	Alabama	27	9	11	0·87	41·0
Grossmann (1940) .	Virginia	59	30	40	0·5	66·7

[1] Based on antitoxin titrations, regarding persons with less than 1/500 unit per ml. as Schick-positive.

Whether immunization does in fact lead to an increase in the proportion of healthy carriers is a difficult question to answer, since so many other factors are concerned. We are not thinking here of what happens in an institution, but in the general population. Clearly if diphtheria is introduced into an institution containing a high proportion of Schick-negative reactors, there is bound to be a temporary rise in the carrier rate, since the majority of those who become infected will not develop clinical diphtheria and will therefore not be removed to an isolation hospital. In the general population, however, one would expect a different sequence. The fewer cases there are as the result of immunization, the fewer contact carriers will there be, and the fewer carriers there are, the less will be the chance of non-immunes developing diphtheria. On the whole, this assumption is borne out by American experience, where in some towns the carrier rate among the general population has fallen to almost negligible proportions following continued mass immunization. The few figures available in this country are in conformity with the findings in America. Thus, before the second world war the carrier rate in London elementary school children varied between about 2·5 and 5·0 per cent. (see Chapter 56). In September, 1942, however, about 9 months after the beginning of the Government's immunization campaign, the carrier rate of virulent diphtheria bacilli was only 0·38 per cent., and in February, 1943, it had fallen to 0·19 per cent. (Report 1944). These figures lend no support to the suggestion that immunization does more harm than good to the herd by increasing the number of healthy carriers.

Prophylactic Passive Immunization.

To meet particular and transitory emergencies, such as the accidental admission to a children's ward of a child suffering from diphtheria, the subcutaneous or intramuscular administration of 500–1,000 units of antitoxin to each of the other children thus exposed to risk has been found an effective method of stopping the spread of the disease. Provided the children have not been exposed to the infected child for more than 24 hours, a Schick test may be carried out, a preliminary reading taken the following day, and inoculation with serum restricted to those giving a positive reaction. Schick-testing, however, seems to us to be of very doubtful value, unless a high proportion of the children are believed to be immune. Many of the susceptibles will not be read as Schick-positive within 24 hours, and if a longer time than this is allowed to elapse before serum is injected, there is a risk of diphtheria developing. On the whole, it is more satisfactory to give serum to every child. So long as refined serum is used, trouble from serum reactions will be slight.

Combined Active and Passive Immunization, and the Control of Institutional Epidemics.

Combined active and passive immunization consists in giving simultaneous injections into opposite arms of serum and of diphtheria prophylactic, followed later by a second dose of prophylactic. Its object is to stimulate an active immunity under cover of a passive immunity. The doses recommended are 500 units of refined serum and 0·3-0·5 ml. of A.P.T., followed in 2–4 weeks' time by a second dose of 0·5 ml. of A.P.T. This method is of particular value when a case of diphtheria develops in a group of susceptible children in a school, hospital, or other institution, when the extent of the distribution of the infecting agent is unknown, or when further introduction of infection is to be anticipated. In these circumstances passive immunization alone, which wears off in 2–3 weeks, may be followed by the appearance of diphtheria cases due to infection from healthy carriers. The combination of active and passive immunization results not only in a transitory protection of the exposed children, but in the development of an active immunity that can generally be relied upon to prevent further cases of diphtheria developing, even in the presence of continued infection. Since, however, there is an intermediate period of relative susceptibility between the wearing off of passive and the development of active immunity, it is wise to swab the entire population and segregate all virulent carriers till a fortnight after the second dose of A.P.T. By this time sufficient active immunity will generally have developed to render almost negligible the risk of further cases occurring. By this method very successful results have been reported by Fulton, Taylor, Wells, and Wilson (1941) in the control of school and institutional epidemics. To the paper by these authors and to one by Downie, Glenny, Parish, Smith, and Wilson (1941) reference may be made for the rationale and application of combined active and passive immunization.

The advantage of this method in conjunction with swabbing of the entire population and segregation of virulent carriers is that school closure is quite unnecessary, and that, apart from the carriers who are segregated for 6 weeks, every child can continue its normal education. Little is to be gained by preliminary Schick-testing of the population, as was recommended by Okell, Eagleton, and O'Brien (1924) in the days before combined active and passive immunization

was shown to be practicable, and valuable time may be lost. Moreover, the danger of diphtheria developing in Schick-negative reactors who are exposed to special risk of infection is by no means remote. On the whole, we think that there is far more to be gained by giving combined active and passive immunization to all children than by immunizing only the Schick-positive reactors. The time and labour expended are also less.

The Standardization of Diphtheria Prophylactics.

The flocculation test of Ramon (1922) provides an obvious method of determining the amount of toxoid in any prophylactic, and Glenny, Pope and Waddington (1925) found that the immunizing value of a modified toxin was closely related to its Lf value. It has, indeed, become a common practice to state the dose of toxoid contained in a prophylactic in terms of Lf units, when the actual dose of toxoid is stated at all, which is not often the case. But it would obviously be preferable, were it possible, to measure the actual immunizing potency of all preparations by direct comparison with some arbitrarily selected standard reagent.

The technical differences involved in such a comparison are, however, very great. For the reasons discussed in Chapter 43, a comparison carried out on a small number of animals is quite valueless; and, in spite of a few reports in a different sense, it is very doubtful whether the accurate standardization of a reagent used for inducing active immunity is as yet within the realm of practical politics.

The relevant regulations in force in Great Britain, under the Therapeutic Substances Act, are as follows:

"Diphtheria Prophylactic shall be submitted to the following tests:

(*a*) *Tests to determine that the specific toxicity of the toxin used in its preparation has been so reduced that it does not exceed the prescribed maximum.*—Five human doses of the Diphtheria Prophylactic under test shall be injected into each of 5 normal guinea-pigs each weighing 250–350 grammes. This injection must not cause the death of any of the guinea-pigs within 6 days following the injection. If all the guinea-pigs injected survive for 6 days but any of them die within 30 days following the injection from the specific toxæmia, one human dose of the Diphtheria Prophylactic under test shall be injected into each of 5 normal guinea-pigs, each weighing 250–350 grammes. This injection must not cause the death of any of the guinea-pigs within 30 days following the injection.

If a batch of Diphtheria Prophylactic is shown by either of these tests to have a greater toxicity than the maximum hereby indicated, it shall not be issued unless and until the toxicity has been so reduced by further treatment that it does not exceed that maximum.

(*b*) *Test for potency as an immunizing antigen.*—A quantity of Diphtheria Prophylactic not exceeding five human doses shall be injected on one occasion into each of 10 normal guinea-pigs; or, alternatively, a quantity of Diphtheria Prophylactic not exceeding one-tenth of a human dose shall be injected into each of 10 normal guinea-pigs on each of two occasions, separated by an interval of not more than 4 weeks. The guinea-pigs shall be tested for immunity to diphtheria toxin, if they have received the single injection hereinbefore prescribed, at a date not later than 6 weeks after injection, and if they have received the two injections hereinbefore prescribed, at a date not later than 3 weeks after the second injection.

The test for immunity may be made by either of the two following methods:

(i) by intracutaneous injection into each guinea-pig of one test-dose of Schick Toxin. If more than 2 out of the 10 guinea-pigs exhibit a positive Schick reaction, the batch of Diphtheria Prophylactic shall be treated as insufficiently potent, and shall not be issued; or

(ii) by subcutaneous injection into each guinea-pig of five minimal lethal doses of diphtheria toxin. If more than 2 out of the 10 guinea-pigs die as the result of this

injection the batch of Diphtheria Prophylactic shall be treated as insufficiently potent, and shall not be issued."

This, it will be noted, is the prescription of limits of toxicity and antigenic efficiency, not standardization in terms of units.

The standardization of diphtheria prophylactics on laboratory animals is not an altogether satisfactory method for determining the antigenic potency for human beings (see Hartley 1934). It is therefore desirable, whenever possible, to control each batch by field observations on children. For this purpose the Schick-conversion rate is suitable (see, for example, Fulton *et al.* 1942).

Other Diseases caused by Corynebacteria

Besides causing infections of the upper respiratory tract, the diphtheria bacillus may sometimes be found as a secondary invader in skin wounds and in lesions of the vagina. Diphtheroid bacilli of various types have frequently been isolated from the sites of pathological conditions in man. They have been cultivated from the conjunctiva in various forms of subacute or chronic conjunctivitis, from the external auditory meatus in cases of ear disease, from the lesions of acne vulgaris, from other lesions of the skin, from the urine in cases of subacute or chronic urethritis, and from lymphatic glands in a variety of conditions, particularly in lymphadenoma. In a few instances, including perhaps acne vulgaris, the evidence suggests that the association is a causative one, but in the vast majority of cases the early claims to have established an ætiological relationship have broken down in the light of subsequent investigations. There is, as yet, no satisfactory evidence that any corynebacterium, other than *C. diphtheriæ*, with the possible exception of *C. acnes*, plays any significant rôle as a pathogenic parasite of man; though various species form an important constituent of his normal bacterial flora (Andrewes *et al.* 1923, Harris and Wade 1915).

Diphtheroid Infections in Animals.

There are a few diseases which occur naturally in animals other than man, and are caused by infection with corynebacteria.

Ulcerative Lymphangitis of Horses and Pseudotuberculosis of Sheep.—Both these diseases are the result of infection with *C. ovis*, or, as it is more commonly called, the Preisz-Nocard bacillus. The characters of this organism and the lesions that it produces in experimental animals have been described in Chapter 17. It may be noted that this organism, unlike *C. diphtheriæ*, is pyogenic and invasive, as well as toxigenic. The exotoxin produced by it differs from the exotoxin of *C. diphtheriæ* both in the character of the lesions produced in experimental animals and in its antigenic relationships. The pathogenesis of the natural disease in horses and sheep seems to be determined mainly by the invasive and pyogenic activities of the causative organism. The part played by the toxin is at present doubtful. It may be noted that pseudotuberculosis of sheep is an important disease, from this standpoint, in certain parts of the world, particularly in Australia (see Bull and Dickinson 1931, 1933, 1935, Dickinson and Bull 1931, Discussion 1934).

Infections caused by C. pyogenes.—This organism is probably the most important and widespread member of the *Corynebacterium* group found in association with animal disease. It is responsible for suppurative processes, including pneumonia, arthritis, and mastitis in cattle, pigs, sheep, and goats. A weak toxin

is formed, which often gives rise to antitoxin production in infected animals; this may be measured by the power of the serum to inhibit the hæmolysis of rabbit cells by the toxin (Lovell 1939). The toxin may be converted into toxoid by treatment with formalin. An alum-precipitated toxoid induces antitoxin formation in animals inoculated intramuscularly, but whether it will prove of prophylactic value under field conditions remains to be decided (Lovell 1941).

Pyæmia in Foals.—Magnusson (1923, 1938) described a pyæmic disease of foals in Sweden due to a pigment-forming organism, which he called *C. equi* (see p. 469). As a rule the disease is characterized by a suppurative bronchopneumonia, with greyish-red pus in the bronchi, and an intense purulent infiltration of the pulmonary lymph nodes. In some cases intestinal ulceration occurs, accompanied by large abscesses in the mesenteric lymph nodes. Though *C. equi* is often present in the submaxillary lymph nodes of pigs, it does not appear to give rise to lesions in these animals (Karlson *et al.* 1940).

Infections caused by C. renale.—This organism appears to give rise to cystitis and pyelitis in cattle (see p. 461).

Pseudotuberculosis of Mice.—This is a natural disease of mice caused by *C. pseudotuberculosis murium*. So far as it is known, no other animal species is susceptible. Since, in this instance, the animal used for experimental purposes has been the natural host, the lesions of the disease have been described in Chapter 17 (p. 461).

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CHAPTER 62

GLANDERS AND MELIOIDOSIS

GLANDERS

NUMEROUS investigators during the nineteenth century brought evidence to show that glanders was infectious; and that the disease in man was the same as that in the horse. The causative agent, however, was not isolated till 1882, when Loeffler and Schütz (Loeffler 1886) succeeded in cultivating *Pf. mallei* from a horse dying of acute glanders. They isolated it in pure culture from the liver and spleen, and produced characteristic lesions in the guinea-pig, rabbit, and field mouse by injection of the bacilli. The organisms were demonstrated histologically in the lesions, and isolated in pure culture.

Epidemiology in Animals.—Glanders is primarily a disease of equine animals. Pigs and cattle are absolutely resistant. Goats, sheep, dogs, and cats sometimes contract the disease naturally; it has also occurred in zoological gardens amongst carnivora, such as lions and tigers, which have been fed on infected horse-flesh.

There are two clinical types of the disease in horses and asses—glanders and farcy. In **glanders,** which may be acute or chronic, the lungs are almost invariably affected. Rounded, greyish, firm nodules, about ½ to 1 cm. in diameter, appear in small numbers—often not more than a dozen or so. They are embedded in the lung tissue, from which they are not easy to enucleate. When recent they have a dirty white centre and a dark red, or sometimes yellow, gelatinous periphery; the central part consists of thick pus. In older nodules the greyish central zone is surrounded by dryish crumbling material, or by a fibrous capsule. In acute cases there may be an actual pneumonic infiltration of the lung. Histologically the young lesions consist of polymorphonuclear cells, surrounded by a zone of congestion. It is stated that, in the older nodules, there is frequently a zone of epithelioid and giant cells around the central necrotic area, and surrounding the whole there is a layer of fibrous tissue. Occasionally calcification may occur (Riegler 1905, Reinhardt 1919). Glanders may invade other organs as well as the lungs, particularly the nasal mucosa and trachea; nodules first appear, which later ulcerate and discharge a greenish-yellow serous fluid, sometimes streaked with blood (Hunting 1908, Hutyra and Marek 1926). The submaxillary glands are often enlarged; subcutaneous abscesses are not uncommon; and in the acute disease nodules are distributed throughout the spleen, liver, and other organs.

In the mule glanders is almost invariably acute, death occurring in 3 or 4 weeks (Mason 1918).

Farcy may result from direct infection of the skin, or it may be a secondary manifestation of glanders. Swellings appear in the skin or subcutaneous tissues, particularly of the extremities and flanks, which break down and ulcerate. The

lymphatic vessels leading from these swellings become firm and enlarged, standing out beneath the skin as hard cords—farcy pipes. These run to the nearest lymphatic glands, which likewise become prominent—farcy buds. Along the course of the lymphatic vessels fresh swellings may appear, so that there may be a chain of nodules connected by firm cords looking not unlike a rosary.

Incidence.—Glanders was at one time widespread throughout Europe, but of recent years, owing to precautionary measures, it has steadily diminished in most countries. It is still prevalent, however, in Russia, where as many as 4 per cent of the horses are said to be infected (Hutyra and Marek 1922). The disease occurs in Asia, Africa, and America; Australia has so far remained free. In Great Britain it was very common previous to the passing of the Glanders or Farcy Order in 1907; since then it has decreased, so that now no more than one or two small epizootic cases are reported (Table 119).

TABLE 119

PREVALENCE OF GLANDERS AND FARCY IN GREAT BRITAIN.

Year.	No. of Outbreaks.	Horses Attacked.	Year.	No. of Outbreaks.	Horses Attacked.
1893 . .	1,381	2,133	1910 . .	351	1,014 [1]
1894 . .	965	1,437	1911 . .	200	504 [1]
1895 . .	964	1,594	1912 . .	172	515 [1]
1896 . .	817	1,294	1913 . .	162	438 [1]
1897 . .	900	1,629	1914 . .	97	286 [1]
1898 . .	748	1,385	1915 . .	49	85 [1]
1899 . .	853	1,472	1916 . .	46	117 [1]
1900 . .	1,119	1,858	1917 . .	25	69 [1]
1901 . .	1,347	2,370	1918 . .	34	98 [1]
1902 . .	1,155	2,040	1919 . .	25	61 [1]
1903 . .	1,456	2,499	1920 . .	15	22 [1]
1904 . .	1,529	2,658	1921 . .	11	45 [1]
1905 . .	1,214	2,068	1922 . .	4	4 [1]
1906 . .	1,066	2,012	1923 . .	9	16 [1]
1907 . .	854	1,921	1924 . .	3	4 [1]
1908 . .	789	2,433 [1]	1925 . .	2	2 [1]
1909 . .	533	1,753 [1]			

[1] Includes horses that reacted to the mallein test, and on post-mortem examination showed glanders lesions.

Both the mule and the ass are very susceptible to glanders, and in them the disease generally takes an acute form. In spite of the high susceptibility of asses, however, these animals do not appear to be as frequently affected as horses and mules. Thus, during the war of 1914–18, Mason (1918) stated that the incidence of glanders in Cairo and Alexandria was approximately 1 in every 4 horses and mules, but only 1 in every 100 asses.

In considering the incidence of glanders, it must be remembered that in many respects the disease resembles tuberculosis. For every clinical case there are a number of latent cases, revealed only by the mallein test or one of the numerous other reactions that are now employed in diagnosis. Occult glanders, in fact, is the rule, not the exception (M'Fadyean 1905). Months may elapse between infection and the onset of symptoms; and in many animals it would appear that the disease may lie permanently dormant, or undergo spontaneous cure. As with

most diseases affecting the respiratory tract, glanders is commonest in cities and in places where large numbers of animals are kept in close contact. During the first 5 years of this century, for example, about 75 per cent. of all cases reported in Great Britain occurred in London (M'Fadyean 1905). The disease used to be especially prevalent in the large studs of horses owned by the Municipal Omnibus Companies; once the infection had been introduced, it spread rapidly throughout the stud. Apart from clinical cases there was an even larger number of carriers, which served to propagate the disease and to render its control extremely difficult.

Mode of Infection.—The mode of infection is not at all clear. Though primary disease of the lung appears to be very common, the belief has grown up that infection does not occur by the respiratory tract. It is difficult to gather from the literature the real incidence of primary glanders of the lung. There are many other diseases that give rise to lesions closely resembling the glanders nodule (Riegler 1905, Joest 1915); and our impression is that in the past many true cases of glanders have been classified under other headings and *vice versa*. Experimentally, most attempts to produce chronic pulmonary glanders *via* the respiratory tract have failed; intratracheal and intranasal injection of the bacilli have resulted in the acute disease. Most workers have therefore concluded that infection does not ordinarily occur by this route. On the other hand, typical pulmonary lesions have followed infection by the mouth (M'Fadyean 1904, Bonome 1906). In animals infected in this way, however, it is usual to find some degree of inflammatory reaction in the intestinal mucosa and submucosa, together with hyperplasia and necrosis of the mesenteric glands. As these lesions are uncommon in horses dying naturally, it is by no means certain that the disease set up in this way corresponds to that occurring spontaneously. Bonome (1906) tries to explain the discrepancy by assuming that the intestinal and mesenteric glandular lesions rapidly retrogress so that in about 2 months only the pulmonary lesions are apparent.

Without discussing this question any further, we may point to the close analogy with tuberculosis, in which much the same arguments have been advanced for and against the respiratory route of infection (see Chapter 59). Whether the infectious material in glanders reaches the lungs by direct inspiration, by absorption through the pharyngeal mucosa, or by passage through the intestine and mesenteric glands, it is impossible to say with certainty. The important point is that the nasal and pulmonary discharges, and sometimes the urine and fæces, of animals suffering from glanders are infectious, and that any other susceptible animal coming into contact directly or indirectly with these discharges is liable to contract the disease.

Reproduction of the Disease.—Farcy may be transmitted by subcutaneous inoculation. Under natural conditions, it is probable that some cases occur by direct infection of the skin—as in grooming—while others are really metastatic infections of pulmonary origin. In nearly every case of farcy, glanders nodules are present in the lungs at necropsy (M'Fadyean 1904).

Glanders may be reproduced in horses, asses, and mules by feeding with cultures of the bacillus, and by subcutaneous inoculation. Sheep and goats are easily infected, but cows and pigs are absolutely resistant. Of laboratory animals the guinea-pig and the field mouse (*Arvicola arvalis*) are the most susceptible to expect mental inoculation; rabbits and dogs are less so; rats, birds, and perhaps white mice, are comparatively resistant. (See Chapter 19.)

Epidemiology in Man.– Glanders in man is uncommon. Robles in 1906 succeeded in collecting reports of 156 cases. It is a protean disease, and probably many real cases that occur are not diagnosed correctly. It may be acute or chronic, and it may be localized chiefly in the respiratory tracts, or in the skin and subcutaneous tissues. In acute glanders there is generally fever, a mucopurulent discharge from the nose, and a degree of prostration out of all proportion to the clinical signs (Bernstein and Carling 1909); a generalized pustular eruption is very frequent. Death invariably occurs in a week or 10 days. In the chronic disease there may be coryza; there may be multiple subcutaneous and intramuscular abscesses, often associated with enlargement of the lymphatic glands and vessels; nodules may form in the mucosa of the respiratory and alimentary tracts, and may break down and ulcerate; necrotic foci may appear in the bones, and in other lesions in the viscera. The disease may remain active for weeks, months, or even years; it is usually but not invariably fatal. Sometimes after apparent recovery the disease may break out again; latent periods up to 20 years have been observed (Bernstein and Carling 1909). The incubation period varies from a few hours to several weeks. (For an account of a case in a veterinary surgeon, see Galger 1913, 1916).

The disease attacks chiefly those who come into close contact with horses such as ostlers, grooms, and coachmen. Infection results most frequently from contamination of a scratch or wound with a glanderous discharge, but primary infection of the nasal mucosa may occur. Several cases of glanders have been reported in laboratory workers; indeed, probably no organism, with the possible exception of *Br. tularensis*, is so dangerous to work with as the glanders bacillus. In the Czernowitz laboratory the disease broke out amongst several members of the staff a few days after the breaking of a centrifuge tube. Numerous other laboratory infections are on record (Bernstein and Carling 1909). When one considers the infectivity of the organism in artificial culture, it is surprising that the disease is not more common amongst those who come into contact with glandered animals. M'Fadyean stated in 1905 that, during the past 12 years, a single firm of knackers in London had dealt with from 800 to 2,000 glandered carcasses; yet not a single case of glanders had occurred amongst the men. There are several statements in the literature suggesting that the virulence of the organism is subject to rapid variation (Gamaléia 1890, Motta 1906, Bernstein and Carling 1909, Dudgeon *et al.* 1918). It may be that the bacilli in chronic lesions of the horse are relatively avirulent to man, in much the same way as they are to the guinea-pig. If this is so, they may be incapable of giving rise to clinical disease. In this connection it may be noted that Babes, while making autopsies on persons who had come into contact with horses and who had died from diseases other than glanders, observed the presence of encapsulated glanders nodules in the internal organs, especially the lungs. This suggests that occult glanders may occur in man as well as in the horse (M'Fadyean 1905).

Diagnosis.

In acute glanders or farcy a microscopical examination should be made of the pus or discharge. Bacilli are fairly numerous in fresh lesions, but may be difficult to find in older ones. Their demonstration in sections of the tissues is often very difficult, and special staining methods have usually to be adopted; the bacilli are more easy to find in smears. Cultures should be put up on agar, glycerine agar,

and potato. If the material is grossly contaminated, it is best to inject it subcutaneously into a guinea-pig, recover the bacillus from the enlarged glands, and inject it intraperitoneally in pure culture into fresh guinea-pigs. In any case the culture should be tested for virulence, and the characteristic Straus reaction noted (see p. 489). Not all strains of *Pf. mallei* are virulent on isolation; sometimes two or three passages have to be made before the typical disease develops (Dudgeon *et al.* 1918). As a rule material from the acute disease in horses proves virulent, but from the chronic disease it is generally avirulent.

The Straus reaction is not diagnostic of glanders; swelling of the testicles and periorchitis may occur after injection of guinea-pigs with organisms of the *Brucella* group, with *Actinobacillus lignieresi*, with Preisz-Nocard's *C. ovis*, with *Pf. whitmori*, and with a few other species less commonly met with.

Mallein Test.—This is used for the diagnosis of latent or chronic glanders in horses. Mallein is prepared by growing the glanders bacillus in flasks of peptone veal broth containing 4 per cent. of glycerol. After 15 to 20 days' incubation the cultures are sterilized in the autoclave, concentrated to one-tenth of their volume in a water-bath, and filtered through paper (Nocard 1895). The resultant product is a syrupy brown liquid; before injection, this crude mallein has to be diluted about 1/10 with sterile water or 0·5 per cent. phenolized saline. The *subcutaneous test* is made by injecting 2·5 ml. of the diluted mallein under the skin of the neck. Sometimes mallein is prepared by simple filtration, without concentration, of a broth culture; in this case 1 ml. is used for injection. A positive reaction is characterized by (1) a local swelling, congestion, and œdema, often very extensive and painful, appearing some hours after the inoculation, increasing for 30 to 36 hours, and lasting for a week or more; it never suppurates; (2) a systemic disturbance, with a rise of temperature, dullness, depression, shivering, anorexia and other symptoms of illness; the temperature rises about the 8th hour, reaches its maximum from the 10th to the 18th hour, and subsides after 36 or 48 hours. The interpretation of the reaction is largely a matter of experience. Generally speaking, a marked local reaction with a rise of temperature of 2° C. or over can be considered positive. A rise of only 1° to 2° C. may be accounted positive if the local reaction is typical; otherwise it should be considered doubtful. If the rise of temperature is less than 1·0° C., the reaction is negative. A rapid rise of temperature, however high, if succeeded by an equally rapid fall, is of no importance. Old horses may fail to react. In doubtful cases the test should be repeated after 4 weeks. In normal horses the injection of mallein causes a very slight reaction; the small local œdematous tumour disappears in 24 to 30 hours.

A definitely positive reaction, even in the absence of clinical disease, may be taken as proof that the horse is glandered. Extensive use of the reaction has shown that, judged by post-mortem findings, a positive result is correct in about 92 per cent., a negative in 96 per cent. of cases. (For interpretation of the reaction see Babes 1891, M'Fadyean 1893, Bonome 1894, Foth 1894*a*, *b*, Report 1915, Mason 1918, Hutyra and Marek 1926.)

The subcutaneous reaction is inapplicable to horses suffering from fever. It has also the disadvantage that it is sometimes followed by the appearance of antibodies in the blood; hence serological tests cannot be used in horses that have been injected with mallein. For these reasons it has largely been abandoned, and replaced by the cutaneous and the conjunctival tests, which apparently have

no effect on the agglutinin titre of the serum (Lovell 1935). In the *conjunctival mallein test* pure mallein is rubbed on the inner surface of the lids with a soft paint brush. In a positive case the reaction appears 4 to 6 hours later, and has a maximum in 8 to 12 hours, and disappears gradually after 24 to 48 hours. There is a secretion of purulent yellow or greenish-yellow material at the inner canthus, and this overflows and runs down the face. The palpebral conjunctiva is red and swollen. In 50–75 per cent. of the cases a rise of temperature occurs (over 103° F.; Reinhardt 1919). In a negative reaction the secretion, if any, is serous or mucoid. The test is contra-indicated in horses suffering from conjunctivitis and from other diseases in which conjunctivitis may develop spontaneously. Horses recently infected, within 4 to 20 days, do not react, nor do horses with chronic glanders, especially if weak or exhausted. On the other hand, the reaction may be positive in horses that have recovered from the disease. In doubtful cases the test may be repeated immediately.

The *cutaneous mallein test* is not used much; but it is of real value, especially when the conjunctival test is inadvisable or yields a doubtful result. Mallein is rubbed into the shaved and scarified skin on the side of the neck. In a positive reaction there is a marked local swelling. A modification of this method, known as the *intradermal palpebral test*, in which the mallein is injected into the cutaneous surface of the eyelid, was employed during the war of 1914–18. In a positive reaction a sensitive œdema of the eyelid is observed about the 9th or 10th hour after injection, accompanied by conjunctival congestion, a mucous discharge from the eye, and closure of the eyelids. The reaction reaches its height between the 24th and 36th hours, and may persist for 3 or 4 days. In normal animals there may be very slight swelling of the eyelid 2 to 6 hours after injection, disappearing about the 12th hour.

Serological Tests.—The **complement-fixation test** is the most reliable laboratory test for glanders. According to Reinhardt (1919) it gives false positives in 4 per cent. of healthy horses, and false negatives in 2 per cent. of glandered horses. According to Poppe (1919), who reports on 2,669 animals, it gave incorrect results in 0·97 per cent. of cases in which glanders was present, and in 2·21 per cent. of cases in which it was absent. It is stated to be sometimes positive in horses suffering from strangles, horse influenza, or petechial fever, and in emaciated animals not suffering from glanders. It may also be positive in normal donkeys, mules, and pregnant mares.

The **agglutination reaction** is less reliable. Though various workers (see Bourges and Méry 1898, Collins 1908, Nevermann 1910) have regarded a titre of 1/1000–1/2000 as practically diagnostic of glanders, it is quite clear that the serum of perfectly normal horses may react to this level (Wilson 1934, Lovell 1935). Much depends on the agglutinability of the strain used and the technique adopted. Without adequate standardization of these factors, the interpretation of the results of different workers is impossible. As in other diseases, the higher the titre, the greater is its significance. The agglutination test, however, should never be relied on exclusively for diagnosis. Repeated tests are of value; if, in a series of tests at intervals of several weeks, the titre remains the same, it is unlikely that the animal is glandered.

The **precipitin reaction** is not trustworthy enough for practical diagnosis, probably because of the presence of non-specific nucleo-protein substances in the

organisms. Sakamoto (1930) has, however, extracted a soluble polysaccharide from cultures of *Pf. mallei*, with which a specific precipitation test is said to be obtainable.

A number of other tests have obtained prominence during recent years, such as the conglutination reaction, and the combined complement-fixation and hæmagglutination reaction or so-called K.H. reaction of Pfeiler and Scheffler (see Poppe 1919). These reactions are said to be of particular value in the diagnosis of the disease in mules, asses, and pregnant mares, the blood of which not infrequently has a marked anticomplementary action.

It will be realized that a large number of tests are available for the diagnosis of glanders. In practice it is unwise to rely exclusively on any one alone. For ordinary routine work the best combination is the complement-fixation and the conjunctival mallein tests. If these give a doubtful result, the other tests may be employed.

In *mice* the mallein test has been used so seldom that it is difficult to gain any idea of its value. Sabolotny (1926) reports a case in which it was positive. The agglutination test is not very reliable, as the serum of normal persons may have a titre up to 1/100. In glanders it may rise to as high as 1/2000 (Galtier 1881, Collins 1908, Dudgeon *et al.* 1918); but on the other hand it may be extremely low. Several cases have been reported in which no agglutination occurred at all, or only up to 1/60 (Gabriélidès and Remlinger 1902, Bernstein and Carling 1909). The complement-fixation reaction is sometimes of value (Watson 1923, Sabolotny 1926). A leucocytosis is often present in acute glanders (Gabriélidès and Remlinger 1902, Bernstein and Carling 1909).

Immunity and Prophylaxis.

There is a considerable amount of evidence to show that horses may recover spontaneously from glanders (Nocard 1900). Only a small proportion of apparently healthy horses that give a positive mallein reaction subsequently develop clinical signs of the disease (M'Faydean 1900). If they are carefully observed, many of them are found to lose their reaction to mallein. It must be noted, however, that the disappearance of the mallein reaction is no proof of cure (M'Fadyean 1901, Bonome 1906). Spontaneous recovery from glanders does not leave the animals with a high degree of immunity. If exposed to natural infection or experimentally inoculated with glanders material, they not infrequently again contract the disease (M'Fadyean 1900, Nocard 1900, Mohler and Eichhorn 1914).

Numerous attempts have been made to produce an active immunity to glanders. Using graded doses of *morvine*—a substance similar to mallein—Babes (1892) stated that he was able to immunize guinea-pigs. Nicolle (1906) likewise claimed to have immunized guinea-pigs (1) by repeated doses of dead bacilli; (2) by repeated injection of living organisms in very small doses; (3) by a single injection of living organisms in a non-fatal dose. But attempts to render horses immune by vaccination or injections of mallein have not been successful (M'Fadyean 1901). Mohler and Eichhorn (1914) vaccinated horses with 3 or 4 doses of dried bacilli; these animals developed agglutinins and complement-fixing bodies, but were still susceptible to natural and experimental infection with glanders.

Mallein has been tried in the curative treatment of horses and of man; some authors report promising results (Babes, V. 1891, Babes, A. 1892, Pilavios 1893, Bonome 1894), but the mass of evidence does not support their conclusions. It must be remembered that both in horses and in man the disease may retrogress

spontaneously, so that it is difficult to estimate the value of any curative treatment.

Serum therapy has been tried in human glanders (Watson 1923), but the number of cases treated is too small to allow of any conclusions.

For the control of glanders in horses the best results have followed the procedure laid down in the Glanders or Farcy Order of 1907. Briefly this provides that every animal with clinical evidence of glanders, or in every case giving a positive mallein test, shall be regarded as infected with glanders and shall be slaughtered. The diseased carcass shall be buried or suitably destroyed, and thorough disinfection of the infected premises shall be carried out. Contacts shall be tested by mallein, and all positive reactors shall be slaughtered. Since the coming into force of this Order in 1908, glanders in Great Britain has diminished steadily, till now it may be considered as completely eradicated (see Table 11.b, p. 1446).

MELIOIDOSIS

The first descriptions of this glanders-like disease in man were given by Whitmore and Krishnaswami in 1912, and Whitmore in 1913, who encountered it at Rangoon. Clinically it appeared to be a septicæmia or pyæmia; post mortem, suppurating or caseous deposits were found in the viscera. A slender, motile, Gram-negative bacillus was isolated from the lesions which reproduced the characteristic disease in guinea-pigs. The disease has been named Melioidosis by Stanton and Fletcher (1921, 1925), and the causative organism *B. whitmori* (*Pfeiffella whitmori* Pinoy). Melioidosis has been reported not only from Burma and British Malaya, but also from Cochin China, Ceylon, Tonkin and South Africa (see Denny and Nicholls 1927, Pons 1927, Pons and Advier 1927, Mesnard, Joyeux, and Gaulete 1928, Souchard and Ragiot 1933, Roten 1933, Mayer and Finlayson 1944). In their monograph published in 1932 Stanton and Fletcher were able to collect records of 83 cases.

In man the clinical course is brief, death generally occurring within 3 or 4 weeks of the onset of symptoms. A chronic non-fatal case characterized by pulmonary and multiple articular lesions has, however, been described by Mayer and Finlayson (1944). At autopsy the commonest findings are localized areas of consolidation in the lungs, composed of aggregations of small suppurating foci, and abscesses or caseous deposits of varying size in the spleen, liver, and sometimes kidneys. In one case in a European recorded by Stanton, Fletcher, and Kanagarayer (1925), the only lesions were two abscesses in the liver with walls consisting of shaggy necrotic material, resembling those found in amœbic dysentery. In several cases the lesions have been confined to the lungs. Histologically they are indistinguishable from those of glanders.

The disease appears to be primarily one of *rodents*. At Kuala Lumpur in the Federated Malay States, Stanton and Fletcher (1925) stated that it had been present first epidemically and later in a sporadic form amongst their stock of laboratory animals during the previous 12 years. The earliest symptom in rabbits and guinea-pigs is a white milky discharge from the eyes and nose; later dyspnœa occurs, followed by death. At post-mortem in acute cases, there are few signs except yellow miliary nodules on the nasal septum. When the disease has lasted for some time, minute caseous nodules of focal necrosis are found in the lungs, spleen, and sometimes in the liver.

Cases of natural infection have been recorded in wild rats, one case in a cat, and one in a horse. Rodents can be infected experimentally by feeding and by injection of the bacilli into the tissues. (See Chapter 19.)

How man becomes infected is not clear. Some cases have undoubtedly followed contamination of the skin, as in morphia addicts, but Stanton and Fletcher (1932) regard the alimentary tract as the most probable route by which the organisms gain access to the body.

Diagnosis of the disease in man during life is not always easy. The organisms can be cultivated from the blood, from abscesses and sinuses, superficial pustules, and the urine. Splenic or hepatic puncture may be useful for obtaining infective material. In one case Martin (1931) isolated the organism from the cerebrospinal fluid. Suspected material should be cultivated on glycerol agar and inoculated into guinea-pigs. One patient tested gave a positive mallein reaction. Agglutination is not of much value, partly because most patients die before agglutinins have had time to develop, and partly because normal agglutinins to 1/80 or over are sometimes present in healthy persons. At autopsy the organisms can be readily isolated from the visceral lesions. (For general review of the disease see Stanton and Fletcher 1932, de Moor *et al.* 1932, Souchard 1932, Couture 1935.)

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CHAPTER 63

CHOLERA

HISTORY.—Though cholera has been endemic in India for centuries, there is no record of its spread to the rest of the world previous to 1817 (Kirchner 1906). Between 1817 and 1823 it invaded many parts of Asia. The second pandemic, 1826–37, was more widespread. Starting in India, it spread to Russia in 1829, and thence to Poland, Germany, Austria, Sweden, and England. Throughout the years 1832–33 the whole of Europe was ravaged. Four thousand deaths occurred in London alone, and 7,000 in Paris. Canada and New York were infected by Irish immigrants fleeing from their native country. The population of Cuba was decimated, and there was a heavy toll of life in Mexico. The third pandemic, 1846–62, again invaded Europe and America. In 1854 the number of deaths in England was 20,000, in Italy 24,000, and in France 140,000. America was infected by way of New Orleans in 1848; thence the disease spread up the Mississippi valley and reached California. In the fourth pandemic, 1864–75, the disease prevailed widely over Asia, Africa, Europe, and America. The fifth pandemic, 1883–96, spread over Egypt, Asia Minor, and Russia; there was a severe outbreak at Hamburg in 1892; and several ports in France, Italy, and Spain were infected. The present or sixth pandemic, which started in 1902, has been confined chiefly to Asia, Egypt, and the Southern countries of Europe. Though the disease has frequently been imported into England and America, since 1873 it has not succeeded in gaining a foothold. With the improved sanitary arrangements of our Western civilization, cholera is being gradually restricted in its field; and it will probably not be long before it is forced back and confined once more to a few endemic foci, such as those in Lower Bengal (Craster 1913*a*, Elkington 1916).

Bacteriology.—At the Berlin Conference of July 1884 Koch (1886*a*) announced his discovery of the causative organism of cholera—the comma bacillus or *Vibrio choleræ*. During the previous year in Egypt and in India he had examined the fæces of 32 patients during life, and the intestinal contents at autopsy of 62 patients who had succumbed to the disease. In not a single instance had he failed to demonstrate the comma bacillus. The organisms were most numerous in the lower half of the small intestine. In acute cases they were present in almost pure culture, but in cases that had lasted longer, and in whom secondary changes had occurred, the vibrios were few and more difficult to find. In a smear from the rice-water stools or the intestinal contents of typical cases, the comma bacilli were arranged with their long axes parallel to one another, presenting a picture similar to that of fish in a stream. The organisms were found in the intestinal contents, in the lumen of the glands, and even between the epithelium and the basement membrane of the mucosa. They were apparently confined to the gut; the mesenteric glands

and the blood were sterile. As a control, Koch examined the intestinal contents of more than 30 cadavers of patients dying from non-choleraic —mostly intestinal —diseases, but he failed to demonstrate the vibrio in a single instance. Though unable to reproduce the disease in lower animals by administration of the vibrio, he came to the conclusion that it was definitely the cause of cholera.

This conclusion was challenged by a number of authorities holding one or other of the multitudinous theories of disease current previous to the bacteriological era. During the next few years their position was strengthened by the discovery of vibrios in all sorts of situations—cheese, dirty well, river and sea water, intestinal abscesses of pigs, diarrhœal faeces of patients with cholera nostras or dysenteric diseases, and other animal and saprophytic sources (see Chapter 22). Since many of these vibrios bore a close resemblance to Koch's comma bacillus, and since their differentiation by means of the morphological, cultural, and pathogenicity tests that were then available was difficult or even impossible, grave suspicion was cast on the ætiological significance of the cholera vibrio.

Later on, however, with the introduction of a more highly specialized technique —especially the agglutination and complement-fixation reactions and Pfeiffer's phenomenon—it was possible to show that the majority of these vibrios differed in one or more essential respects from the comma bacillus. Even then the confusion was not altogether cleared up; for vibrios were isolated from persons who had been in contact with cholera patients, and from water supplies in areas where cholera was epidemic, which, though conforming to the true cholera type in most respects, yet failed to agglutinate with a specific serum. Intensive study of these inagglutinable strains has rendered it probable that many of them belong to the cholera species, but that through the effect of environment they have undergone slight changes which render their identification when first isolated peculiarly difficult. By frequent subculture in the laboratory and passage through the peritoneal cavity of guinea-pigs or mice many of these strains appear to have recovered their agglutinability (Zlatogoroff 1909, Craster 1914, Taylor and Ahuja 1935).

Koch's observations have been repeatedly confirmed in all parts of the world. In large outbreaks of cholera, the comma bacillus has been found in nearly every case; and the further study of the vibrio has left little doubt in the minds of most observers that it is the specific cause of the disease. It is only fair, however, to point out that not everyone accepts this unitarian view of the origin of cholera. The place of the El Tor vibrio, in particular, has for long given rise to discussion, which the observations of de Moor (1938) in the Celebes outbreak of 1937 have but served to accentuate. In this outbreak de Moor isolated hæmolytic agglutinable vibrios from the patients themselves and from a number of healthy contacts. Van Loghem (1938), who examined de Moor's strains, concluded that they were identical with El Tor vibrios, and being unable to deny their pathogenic rôle concluded that the Celebes outbreak was not true cholera, but a specific enteritis caused by the El Tor bacillus. Otten (1939), however, found that the hæmolytic activity of the Celebes strains was considerably less than that of true El Tor strains, and therefore left the real nature of the Celebes outbreak undetermined. The differentiation of the El Tor from the true cholera vibrio is not always easy, but most recent workers who have given their attention to this problem (see Vassiliadis 1937, Venkatraman, Krishnaswami, and Ramakrishnan 1941) are of the opinion that the El Tor vibrio is not a cause of true cholera.

Bacteriologically, the disease is essentially due to the growth, often in almost pure culture, of cholera vibrios in the intestine. On their autolysis the organisms liberate toxic bodies that have an extremely irritating effect on the intestinal tract, leading to severe and repeated purging, exhaustion, and death.

Experimental Reproduction of the Disease in Animals and Man.—Natural cholera in animals is unknown, but there are certain experimental procedures by which a disease closely resembling cholera can be reproduced in guinea-pigs and rabbits.

Using dogs and rabbits, Nicati and Rietsch (1884) found that the direct injection of comma bacilli into the duodenum was followed by a fatal disease, characterized by the presence of vibrios in large numbers in the small intestine. Koch (1886*b*) confirmed this observation on guinea-pigs, but pointed out that the frequency of a fatal termination depended on the amount of trauma accompanying the injection. He tried to infect the animals by the mouth; and in order to enable the vibrios to pass through the stomach unharmed, he rendered the gastric contents alkaline by the administration of 5 ml. of 5 per cent. sodium carbonate solution. Twenty guinea-pigs treated in this way were shortly afterwards fed with 10 ml. of a broth culture of cholera. Only one died—an animal that had recently aborted. This suggested to Koch that the relaxation of the animal's intestine might have been responsible for the infection. Into his next batch of 35 guinea-pigs, therefore, besides giving the sodium carbonate and the cholera culture, he injected 1 ml. of *Tinct. opii* intraperitoneally to paralyse the gut, and to prolong the stay of the vibrios in the small intestine. The result was that 30 of the animals died, displaying before death weakness of the hind limbs and collapse. At necropsy, the small intestine was deeply injected and filled with a flocculent colourless fluid, containing the vibrios in almost pure culture.

Metchnikoff (1894), working at first with the Massauah bacillus, and later with a true cholera strain, was able to convey the disease to sucking rabbits by rubbing the vibrios on the mother's teats. Diarrhœa usually followed, and about half of the animals died—in 2 to 5 days. What was even more interesting was that if the animals, shortly after they became ill, were put alongside litters of normal rabbits, many of the latter became infected. Sanarelli (1921), from experiments on very young rabbits, concluded that the vibrios were absorbed by the bucco-pharyngeal mucosa, passed through the cervical lymphatic glands, entered the blood stream, and were excreted into the cæcum and colon, whence they ascended the small intestine, and gave rise to the typical lesions of the disease. After the rabbits were 10 days old they could no longer be infected by the mouth. In man, however, tissue invasion appears to be very uncommon.

Adult rabbits can also be infected experimentally, though by a different route. Thomas (1893) found that if they were injected intravenously with living vibrios, most of them died within 36 hours; diarrhœa was present as a rule only in those that lived longer than 24 hours. Post mortem, however, in all the animals there was a creamy, soup-like fluid in the duodenum, and rice-water in the jejunum and ileum, containing vibrios in large numbers. Small ecchymoses of the mucosa were also seen. These findings were confirmed by Issaeff and Kolle (1894), who pointed out that the most typical picture occurred in those rabbits which survived for 3 or 4 days after injection. At the post-mortem examination of these animals, as well as the changes already described, most of the epithelium was found to be detached, so that the internal surface of the small gut was lined by the basement membrane. Isolated patches of epithelium were packed with vibrios. Of 35 rabbits inoculated with doses varying from 1/20 loopful to 9 loopfuls of an 18-hours' agar culture, 27 died.

In man there have been numerous cases of laboratory infection, both in-

tentional and unintentional, resulting from cholera cultures. Koch (1886) recorded the case of a medical man who attended a bacteriological course in Berlin, and developed cholera. Since there was no cholera at the time either in Berlin or the rest of Germany, it is practically certain that he contracted his infection in the laboratory. Dr. Oergel of Hamburg infected himself with the peritoneal exudate of a guinea-pig that had been injected with cholera vibrios; a drop spurted up and entered his mouth. The following day he was ill, and a few days later he died in coma. There was no cholera in Germany at that time. Pfeiffer and Pfuhl (see Kolle 1894) both contracted infection in the laboratory. Pfeiffer, who infected himself at Berlin while making animal experiments with a strain freshly isolated from a cholera case in Hamburg, developed a sharp attack: vibrios were found in his fæces and persisted till the 33rd day of the disease. Agglutinins to *V. choleræ* were demonstrated in his serum. Pfuhl had only a mild attack, vibrios persisting in his fæces for 8 days. Numerous other instances are recorded of similar laboratory infections (Kolle and Schürmann 1912).

Amongst the intentional cases, the best known are those of von Pettenkofer and Emmerich. Doubting the value of Koch's work, both these observers decided to test the effect of the vibrio on themselves. After a preliminary dose of sodium bicarbonate, they drank some water to which a small amount of fresh cholera culture had been added. Von Pettenkofer suffered from diarrhœa only; but his less fortunate companion developed a severe attack of cholera. As well as having diarrhœa with typical rice-water stools, he passed into a profound state of toxæmia with suppression of urine and the *vox cholerica*. Many attempts to produce artificial infection in man have, however, been fruitless. Metchnikoff (1893) carried out a number of experiments on himself and others, sometimes with positive, but more often with negative results. So far as the ætiology of the disease is concerned, negative experiments are, as Koch pointed out, of little value. We do not know all the factors that are necessary for the reproduction of the disease, and quite often the conditions in individual experiments must be unsuitable. The experiments that Nature provides on a large scale are far more convincing.

The Toxins of Vibrio Choleræ.—These have already been described in Chapter 22.

Epidemiology.

In India cholera is most prevalent during the hot weather. Thus in Lower Bengal the maximum incidence is reached from February to May; in the Punjab from May to October (Rogers 1911). The disease is commoner in men than in women. The case mortality is lowest in the 11–20 age group, and increases with age; in the figures cited by Rogers the case mortality in patients between 11 and 20 years of age was 51·3 per cent., in those over 50 years of age it was 73·7 per cent. The effect of atmospheric conditions seems to vary in different parts of India; those who are interested will do well to study the very careful statistical analyses made by Russell and Sundararajan (1928). The disease in India occurs with a certain periodicity, and by calculations based on the relevant data it is said to be possible to predict an epidemic 2 or 3 months ahead of its occurrence (Russell and Sundararajan 1926–27).

The true home of cholera is the delta of the Ganges. Here the disease has occurred year after year for centuries. Previous to 1817 it had apparently never spread widely from this area, but since that year it has manifested an increasing

disposition to invade not only other parts of India, but almost the whole world. The disease now recurs so constantly in certain areas, such as the West Indies, Southern China, the Philippines, Persia, Arabia, and South-East Russia, that these must be regarded as endemic foci. In other areas it usually dies out, though it may be reimplanted from time to time. In India alone the number of deaths from cholera during the 10 years 1898–1907 was 370,000 or 1·64 per 1,000 of the population. It is the low-lying alluvial tracts and the seaports that are most invaded; on high land the disease spreads less readily.

Mode of Spread.—There are many ways in which infection can be transmitted from the sick to the healthy person. A study of the epidemiology of cholera indicates, however, that outbreaks can be divided into those dependent on (1) water-borne infection, and (2) case or carrier infection.

The classical example of a *water-borne* outbreak is the Hamburg epidemic of 1892. Hamburg, and two of its suburbs—Altona and Wandsbeck—were provided each with a different water supply. Hamburg drew its water from the river Elbe at a point above the city, and did not filter it. Altona drew its water from the Elbe below Hamburg, after the fluid refuse and fæces of nearly 800,000 people had been poured into it, but it took the precaution to instal a highly efficient sand-filtration plant. Wandsbeck received its supply from a relatively non-polluted lake, and likewise filtered it. On August 16, 1892, a case of cholera was reported in Hamburg. Others occurred in rapid succession, till by the end of the month there were about 1,000 fresh cases a day. When the epidemic came to an end, on October 23, the figures were: Cases 18,000; deaths 8,200. The incidence of cholera in Altona and Wandsbeck was in comparison very low. Altona reported 516 cases, but there is reason to believe that most of these acquired their infection in Hamburg. On the Hamburg-Altona frontier the line of demarcation between the two towns was very irregular, and there was much overlapping of the water supplies. It was observed that in the same street the houses that were supplied with the Hamburg water were invaded by cholera, whereas those supplied with the Altona water were spared. The Elbe was probably infected originally from the excreta of cholera patients on board the numerous barges anchoring opposite the Hamburg water intake. During the outbreak, cholera vibrios were isolated from the river not far below the mouth of the main Hamburg sewer (Koch 1894). There is here a perfectly clear demonstration of the ability of water to convey the cholera vibrio, and of the efficacy of sand-filtration in protecting a town against a highly polluted source of water. Numerous other outbreaks are on record in which the water supply has become infected and has led to the development of cholera.

Epidemics originating in this way are generally explosive; the ascending limb of the incidence curve rises very steeply; the epidemic soon reaches its maximum, and with the cleansing of the water begins to fall. The decline is, however, less steep than the ascent, probably because a number of contact infections occur that are not directly dependent on the water-borne infection at all, and therefore continue to arise even after the water is clean again.

The second method of infection—*case or carrier infection*—is the one by which the disease is spread from place to place, and attains its widespread distribution. Infected material is conveyed from the sick to the healthy person, either by water, food, or infected linen. Milk, raw fruits and vegetables, and other uncooked food are all able to serve as media for the transference of the vibrio. Clothing, especially

linen, if kept moist, can retain its infectivity for several days or even weeks. Numerous experiments have been made to test the survival time of the cholera vibrio under natural conditions.

Greig (1913–14*b*) in India stored the rice-water stools of 94 patients in a dark cupboard at room temperature, taking care to prevent evaporation. The average life of the vibrio was 7 to 9 days in the cooler weather, and 1 to 2 days in the hot weather. The longest survival time was 17 days. Gamaleïa (1893) found that on linen strips kept moist in a water-saturated atmosphere the vibrios lived for about 5 weeks; when the strips were dried in a desiccator, they died in 17 hours. Dried on silk threads the vibrios rarely live for more than a few hours, but sometimes they may survive for 3 or 4 days (Kitasato 1889). Berckholz (1889), however, found that on threads dried in air they might live for as long as 30 days. In water *V. cholerae* remains viable for a variable time. Houston (1909), who added cultures of the vibrio to raw river water, found that 99·9 per cent. of the organisms were dead in a week; none survived longer than 2 weeks. Haffkine and Simpson (1895) in Calcutta brought evidence to show that the survival time of the vibrio in water was less than 5 weeks. Some natural waters, such as the Jumna and the Ganges (Hankin 1896), appear to be unfavourable to its survival. Probably much depends on the purity of the water. In spring water the vibrios lived for 30 days, in Berlin sewer water for 6 to 7 days, and in cesspool water for less than 24 hours (Koch 1886*b*).

Speaking generally, we may say that heat and desiccation are rapidly destructive; but that under suitable conditions of moisture and temperature the cholera vibrio may survive outside the body for a considerable time.

Patients who have recovered from cholera may continue to excrete the vibrio —often very irregularly—for some weeks. As a rule about 90 per cent. become free from infection within a fortnight, and 99 per cent. in a month. But occasionally the vibrios may persist for 3 to 4 months, or even—as in one recorded case —for a year. It is not only convalescents, however, who are infective; healthy persons who have been in contact with cholera cases may become infected and excrete the vibrios without manifesting any sign of the disease. Sometimes cholera eventually develops in these healthy carriers—possibly owing to an alimentary indiscretion—and if this occurs some time after their contact with cholera patients it may be very puzzling to trace the source of their infection. Munson (1915), for example, discovered three healthy carriers in a prison in Manila. These were isolated and examined daily. Two of them, after having been carriers for 16 and 17 days respectively, developed cholera, and the third developed it after 18 days and died in 8 hours. As Munson points out, this man might have travelled half-way round the world scattering his infection broadcast during his 18-day period as a carrier, and died of true cholera in a place many thousands of miles from any other source of infection. These cases are probably exceptional, but there is evidence to suggest that many persons do not develop the disease clinically for some hours—24 or more—after the vibrios are demonstrable in the fæces (Crendiropoulo 1912). It is probable that the cholera vibrio often survives in the gall-bladder, and from there is excreted into the intestine. Greig (1913), who examined the bile of 271 fatal cases of cholera, isolated the specific vibrio from 80 of these, and found evidence of cholecystitis in 12 of the 80 positive cases. Similar results are reported by Schobl, Kulescha, Hunter and others (see Elkington 1916). Sometimes it is excreted in the urine (Greig 1913–14*a*).

The spread of contact infection may be aided to some extent by flies. Cholera vibrios have been isolated from flies taken in infected houses, from flies caught in a post-mortem room in which cholera corpses had been examined, and from the

feet of flies caught 17 hours after their experimental contamination (Elkington 1916).

Contact infection is responsible for the usual *chain-spread* of cholera. Cases occur in different localities, often widely separated from each other, exhibiting a regular sequence of infection. With the rapidity of modern transport it is often very difficult to ascertain all the links in a given chain. When it is remembered that infection may be carried and transmitted by apparently healthy persons, the magnitude of this difficulty will be appreciated. An excellent example of this chain-spread is afforded by the Hamburg epidemic of 1892. This, as we have already stated, was due to an infected water supply; but from Hamburg the disease was carried by infected persons to nearly 300 places in Germany and other countries.

Often the presence of a carrier in a community is rendered probable by the development at intervals of clinical cholera cases for which no obvious source of infection is recognizable. The production of these "cholera nests," as they are called, is particularly frequent in the East, where the sanitary conditions are poor.

The two methods of infection described may be, and frequently are, combined. In the suburbs of Calcutta, for example, the tanks, which supply a large proportion of the native population with water, are freely open to pollution from cholera cases, so that whenever a tank belonging to a given household becomes contaminated, a small outbreak may result. Since the water in these tanks is used not only for drinking, but for washing, bathing, and sewage disposal, it is not surprising that they are frequently responsible for the conveyance of infection.

Diagnosis.

The bacteriological diagnosis of cholera may be very easy, or remarkably difficult. Here we can indicate only the outline of the methods to be employed. (For further details see Koch 1886*a*, 1894, Ellermann 1904, Crendiropoulo 1912, Craster 1913*a*, *b*.)

The fæces or intestinal contents should be examined microscopically. A flake of mucus, for preference, should be spread on a slide and stained with dilute carbol-fuchsin. If comma bacilli are present in pure or almost pure culture—especially if they present the typical fish-in-stream appearance—a provisional diagnosis of cholera can be made directly. By this means alone Koch (1894) was able to diagnose about 50 per cent. of the specimens sent for examination to the Institute for Infectious Diseases at Berlin. If, however, other bacilli are present, it is advisable to suspend judgment till further examination has been made.

The fæces should be transported to the laboratory as rapidly as possible. If more than a few hours are likely to elapse before their reception, they may be put into a preserving solution such as the borate saline mixture described by Venkatraman and Ramakrishnan (1941). On arrival at the laboratory they should be seeded at once into a tube of ordinary peptone water (1 per cent. peptone, 0·5 per cent. NaCl). In this medium, cholera vibrios are able to grow more rapidly than other intestinal bacilli. Multiplication occurs chiefly at the surface, where a thin pellicle forms in the course of a few hours. After 6 to 12 hours' incubation at 37° C., a loopful taken from the surface should be filmed, and if vibrios are present, a fresh tube of peptone water should be seeded and an agar plate streaked with a loopful taken from the surface of the culture. Alternatively a selective medium, such as Dieudonné's (see Vedder and van Dam 1932), the phenolphthalein

starch medium described by Yen (1932-33), or the sucrose mannitol sulphate bismuth alcohol phenol red agar medium of Wilson and Reilly (1940) may be used. After 12 to 18 hours' incubation, the peptone water culture should be tested by the addition of pure sulphuric acid for the cholera-red reaction. The colonies on the agar plate should be examined after 12 hours, and mixed. If they consist of vibrios, 1·5 mgm. (about a loopful) of culture should be rubbed up in 1 ml. of broth and injected intraperitoneally into a guinea-pig. At the same time 2 or 3 tubes of broth should be seeded with single colonies, and incubated at 37° C. After 3 days they should be tested for haemolysin production by the addition of 1 ml. of culture to an equal quantity of a 5 per cent. suspension of washed goat or sheep red blood cells; this mixture should be incubated for 2 hours at 37° C. and kept overnight in the cold before reading. A suspension of colonies on the agar plate should be made in 0·2 per cent. formol, and tested against an agglutinating serum containing the specific cholera O (sub-group I) antibody (see Gardiner and Venkatraman, 1935); living vibrios may be used instead, but not boiled or chloroformed suspensions. For practical purposes the isolation of a non-haemolytic vibrio with the specific O antigen of sub-group I may be regarded as diagnostic of cholera. Vibrios having the O antigen of sub-group I, but haemolysing sheep or goat red cells, belong to the El Tor group. Not too much weight, however, should be placed on the hæmolytic or non-hæmolytic activity of the organism, since the ability to produce an active hæmolysin seems to depend on a number of factors which are not always easy to control (see Chapter 22).

If the cholera vibrios are scanty, and particularly if they are being sought for in water, it is advisable to incubate the material in a sulphite bismuth enriching fluid medium such as that described by Read (1939) or Wilson and Reilly (1940) before plating out. A higher proportion of successful isolations may be expected by such a method (Seal 1939). The final identification of the organism should be made by the absorption of agglutinins test and by Pfeiffer's (1895) reaction. For this 4 guinea-pigs are required. (1) 1 loopful of an 18 hours' agar culture at 37° C., suspended in 1 ml. of broth, is mixed with 0·001 ml. of a high-titre cholera antiserum and injected intraperitoneally. (2) The same inoculum, with 0·002 ml. of antiserum. (3) The same inoculum, with 0·01 ml. of normal serum. (4) 1 loopful of culture alone is injected. The 3rd and 4th animals act as controls. Immediately after injection, and 20, 40 and 60 minutes later, a drop of peritoneal exudate is removed by a fine Pasteur pipette from each of the four animals, and examined in a hanging-drop preparation. If the reaction is positive, in animals 1 and 2 the vibrios will lose their motility, swell up, and undergo granular degeneration; in animals 3 and 4 the drop will be seething with actively motile, comma-shaped vibrios. The reaction may alternatively be carried out *in vitro*. Pfeiffer's reaction is highly specific. If a mixture of *V. choleræ*, and *V. metchnikovi*, for example, is added to an immune anti-cholera serum and injected into a guinea-pig, only the cholera vibrios undergo lysis; the others are unaffected, multiply, and kill the animal.

During an epidemic of cholera the bacteriological diagnosis can usually be made by simple microscopical and cultural examination, supplemented, if necessary, by the cholera-red and the agglutination reaction. But in non-epidemic times, or at the beginning of an epidemic, especially in a country usually free from cholera, the diagnosis should never be made till all the tests described have been performed and found positive.

The identification of vibrios from carriers or cholera contacts, and from non-human sources such as water or milk, is often very difficult, and has to be performed with the utmost care. Vibrios are often found under these conditions (Dunbar 1893, Kutscher 1893, 1895, Gotschlich 1895, 1906, Haffkine and Simpson 1895, Neufeld and Haendel 1907, Ruffer 1907, Zlatogoroff 1909, Crendiropoulo 1912, Craster 1913*a*, *b*, 1914, Jermoljewa 1926) that bear an extremely close resemblance to the cholera vibrio, and can be differentiated from it only by intensive study. Even in cholera stools, antigenically atypical forms, such as those described by Chen (1932–33), Yang and White (1934), Aoki and Oshiro (1934), and White (1935), are often found. In the examination of such vibrios, attempts should be made by repeated subculture and intraperitoneal inoculation of mice or guinea-pigs to bring about a change to the typical antigenic form. Most non-cholera vibrios are harmless to guinea-pigs, but the pathogenicity test cannot be relied on exclusively.

In the examination of suspected convalescents, besides looking for the vibrio in the fæces, it is well to test the serum for agglutinins and bactericidins. When a large-scale investigation has to be carried out in the search for carriers, a rectal swab should be taken from each person and inoculated into peptone water. Subcultures and plates are made from this after 6 to 9 hours' incubation. Using this method Craster (1913*b*) at the Quarantine Station, New York, examined nearly 27,000 immigrants in 4 months, and identified 31 as carriers.

Prophylaxis and Treatment.

The prevention of cholera is similar to that of typhoid. Owing to the serious nature of the disease stringent precautions have been taken, particularly at seaports, to prevent its introduction into countries usually free from cholera and its subsequent dissemination. (For an account of these see Elkington 1916.)

Vaccination.—Ferran (1885) in Spain was the first to attempt vaccination against cholera. He injected small doses up to about 0·5 ml. of a living broth culture subcutaneously; a severe local and general reaction followed. The results were not satisfactory; and the work was suppressed by the Government.

Haffkine, a pupil of Pasteur's, then took up the problem. He (1892) prepared two living vaccines: (1) consisting of vibrios whose virulence had been attenuated by frequent cultivation in a constantly aerated atmosphere; (2) consisting of vibrios whose virulence had been exalted by 20 to 30 passages through the peritoneal cavity of guinea-pigs. These vaccines were used on a fairly large scale in India; sometimes only the first vaccine was given, sometimes both. The figures have been published by Haffkine (1895*a*, *b*, 1913), and critically discussed by Kolle (1896*b*). It is impossible here to give the results in tabular form. The injections were made on numerous tea estates, in regiments, gaols, and families. The numbers of inoculated and uninoculated were often unequal; the two sets were unevenly exposed to risk of infection; in some instances the inoculation was performed during the progress of the epidemic; in others the disease did not break out for a year or more after inoculation; and the cases that did occur were too few to afford a correct idea of the comparative immunity of the two groups. Haffkine's statistics have been carefully examined by Greenwood and Yule (1915), who point out the variability of the results of inoculations performed in different areas. They conclude, however, that certain of the results establish a presumption in favour of the value of Haffkine's inoculation.

Later (1911, 1913), Haffkine abandoned his living vaccines, and recommended

the use of his vaccine ii, killed by heat at 50° C. for a few minutes, and preserved with 0·5 per cent. phenol. Kolle (1896*a*) was the first to introduce the killed vaccine. By experiments on himself and his colleagues, he showed that the injection of 1 10 to 1 5 of a 24-hours' agar culture, killed by chloroform vapour or heat, stimulated the production of specific bactericidal antibodies. His vaccine has been employed on a fairly large scale by Murata (1904) in Japan, and by Savas (1914) in Greece. The significance of Murata's results is very doubtful, since only one inoculation was given, and there is some doubt as to the homogeneity of his statistics (see Greenwood and Yule 1915). Savas employed anti-cholera vaccination in the Grecian Army during the second Balkan War. As the greater part of the inoculations were made during the progress of the cholera epidemic, it follows that the exposure to risk of infection was different amongst the inoculated and the uninoculated. For this reason statistical analysis of most of Savas' figures is not likely to afford much information as to the value of vaccination. An exception must be made for the Sanitary Corps, nearly every member of which received two inoculations before the disease broke out. A comparison made between the incidence of cholera among the members of the Sanitary Corps and among the combatants is recorded in Table 120 (taken from Greenwood and Yule 1915).

TABLE 120

	Not Attacked.	Attacked.	Total.
Sanitary Corps	2,884	13	2,897
Combatants	112,613	2,192	114,805
Total	115,497	2,205	117,702

$\chi^2 = 32{\cdot}79$. P = less than 0·0001.

The value of χ^2 is high; and the probability that the difference in the attack rates between the two groups is attributable to chance is much less than 1 in 10,000 (see p. 1551). Greenwood and Yule (1915) conclude from these results that "anti-cholera inoculation . . . is a prophylactic step of importance, although an exact statistical measure of the degree of relative immunity conferred cannot be provided."

Kolle's vaccine—a 24-hours' agar culture killed by heat at 55° C. for 1 hour, and preserved with 0·5 per cent. phenol—has now replaced all others. The standardization and dosage of this vaccine vary in different parts of the world. Two doses, each of 2,000 million organisms, at an interval of at least a week, should be given. If only one dose is practicable, 4,000 to 8,000 million organisms may be recommended (Dzen and Yu 1936). According to Griffiths (1944) antibodies, demonstrable by a mouse protection test, appear in the serum one week after vaccination.

Bacteriophage Treatment.—Administration of bacteriophage is now being tried both prophylactically and therapeutically in the treatment of cholera. The data so far collected are scanty and refer to native populations, amongst whom the collection of exact records presents peculiar difficulties (see Morison 1932, Asheshov *et al.* 1930, Asheshov 1933). What is usually regarded as the ideal method—

that of treating alternate cases—is not only generally impossible but may be undesirable, since under primitive conditions of sanitation rapid transference of the bacteriophage from treated to untreated persons is likely to occur. Comparisons have usually to be made (1) between a group of treated and another of untreated patients in any given epidemic; unless the two groups are practically identical in age, sex, time of exposure, risk of exposure, and other relevant factors, such a comparison may yield very misleading results; (2) between the mortality in treated cases in one epidemic and that in untreated cases in a previous epidemic; since the case mortality in different epidemics is subject to considerable variation altogether apart from any active interference on the part of man, this method is likewise unsatisfactory. For the moment, therefore, we must reserve our judgment on the value of bacteriophage treatment. We may note, however, that in cholera the bacteriophage ought to be acting under probably more favourable conditions than in any other disease. The organisms are multiplying in the fluid contents of the intestine, not in the intestinal wall, as in dysentery and enteric fever, where the action of the bacteriophage is likely to be nullified by the tissues. In view of the favourable light in which this method is regarded by many clinicians, it seems probable that it does have some beneficial effect on the course of the disease, but in this, as in other similar problems, we must await the collection of adequate statistical material before coming to a definite decision.

Serum Therapy.—Antisera to cholera are readily prepared by the injection of goats or horses with dead, followed by living, vibrios. The resultant serum has a marked prophylactic effect when tested by the intraperitoneal method in guinea-pigs, but unfortunately its therapeutic powers are low. Injected more than an hour or so after the vibrios, it fails to save the animal's life (Pfeiffer 1894). This is doubtless because the antibodies in the serum are chiefly bactericidal, and are unable to neutralize the toxins liberated by the disintegration of the vibrios. For the treatment of cholera cases, a serum is required that has a high anti-endotoxic titre. Several attempts have been made to produce such a serum. Macfadyen (1906) used an extract of young agar cultures ground up in liquid air for immunizing rabbits and goats. Tested on guinea-pigs 0·5 ml. of the rabbit's serum, after inoculation of toxin, protected against 8 lethal doses, and 0·002 ml. of the goat's serum protected against 3 lethal doses. Schurupow (1909) immunized horses with an alkaline extract of 2-days' cholera cultures, and apparently succeeded in obtaining an anti-endotoxic serum. Unfortunately these sera have not been used on a large scale in practice, and it is impossible, in spite of the favourable reports (Savas 1914) that have been made on them, to estimate their value in treatment.

Chemotherapy.—The information so far available on the value of the sulphonamide group of drugs in the treatment of cholera is promising. Hopeful results have been reported by Chopra, de Monte, Gupta and Chatterji (1941) and by Huang (1944) from the use of sulphaguanidine. During an outbreak in Kweilin, Huang gave adult patients an initial dose of 3 gm., followed by six 1 gm. doses at 2-hourly intervals, and 1 gm. doses every 4 hours for one to two days. The patients are said to have improved within 3–4 hours after taking the drug; the stools became negative in 8 hours. Of 22 patients treated, only one died. Previously, patients treated in Kweilin by the routine method of hypertonic saline, kaolin, potassium permanganate and so on had a case mortality of 26 per cent.

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CHAPTER 64

MENINGITIS

Introductory

Meningitis is an inflammatory affection of the membranes surrounding the brain and spinal cord, which occurs sometimes as a primary disease, and sometimes secondarily to disease of some other part of the body.

Prior to the advent of bacteriology the tuberculous form was recognized, the cerebrospinal form when it occurred in epidemics, and the post-basic form in children.

The discovery of the tubercle bacillus by Koch in 1882 provided a bacteriological means of differentiating the tuberculous from other forms of meningitis. Pneumococci were shown by Fraenkel (1886), Foà and Bordoni-Uffreduzzi (1886), and others to be responsible for the meningitis which occasionally complicates lobar pneumonia. In 1887 Weichselbaum of Vienna published his classical paper on the finding of a Gram-negative diplococcus in six cases of acute cerebrospinal meningitis; this organism he named the *Diplococcus intracellularis meningitidis*. The same year his work was confirmed by Goldschmidt (1887) and by Edler (1884–88). Jaeger (1895), in describing the differences between the pneumococcus and the meningococcus, assigned to the latter characters which it does not possess, and numerous papers were published during the following 10 years dealing with the differences between his description and that of Weichselbaum (Scherer 1895, von Hibler 1896, Heubner 1896, Kiefer 1896, Kister 1896, Still 1898, Councilman *et al.* 1898, Faber 1900, Jaeger 1903*a*, *b*, Albrecht and Ghon 1901, 1902, 1903). The correctness of Weichselbaum's observations was finally established by the extensive studies of von Lingelsheim (1905*a*, *b*) during an epidemic of cerebrospinal fever in 1904–5. Since that date the *Diplococcus intracellularis meningitidis* of Weichselbaum, or, as it is now called, *Neisseria meningitidis*, has been recognized as the causative organism of the disease. Still showed in 1898 that the post-basic meningitis of children is due to a Gram-negative diplococcus apparently identical with Weichselbaum's, and subsequent work has shown that the two organisms are the same.

Other forms of meningitis will be dealt with at the end of the present chapter

Cerebrospinal Meningitis

This is an infectious disease characterized by inflammation of the meninges, particularly at the base of the brain. It may be acute or chronic, and may occur sporadically or epidemically. The causative agent is the meningococcus. The disease can be reproduced experimentally by the intrathecal inoculation of virulent meningococci into monkeys and rabbits (see Chapter 23). There is reason to

believe that the pathological manifestations are due mainly to the presence of endotoxic substances, which are contained in the bacterial cells and are liberated when these undergo autolysis.

Epidemiology.

I. History and Incidence.—The first definite knowledge that we have of cerebro-spinal fever is due to Vieusseux, who in 1805 described an outbreak of the disease at Geneva. In the following year it appeared in Medfield, Massachusetts, and during 1806-07 it attacked the Prussian Army. Since then it has invaded most countries in the world, rising at one time to epidemic proportions, subsiding at other times to remain dormant for long periods, but inevitably reappearing in outbreaks of greater or less severity. The disease has, in fact, shown a gradual increase, both in its geographical range and in the number of persons it has attacked. Thus, according to Hirsch (1886) from 1805 to 1830 the disease was most prevalent in the United States; from 1837 to 1850 France was attacked most severely; from 1854 to 1874 epidemics occurred both in Europe and in America, and from 1875 down to the present time not only these two continents but Asia, Africa, and Australia have been invaded (Low 1916).

The first undoubted case reported in Great Britain occurred in 1830. From that date the disease remained endemic in this country, giving rise to occasional small outbursts. Our knowledge of the disease previous to 1912, the year in which compulsory notification was introduced, is largely furnished by the death returns; these record a gradual rise from 1900, when 9 deaths were recorded in England and Wales, to 1911, when 134 were recorded. In 1907 there was an outbreak of epidemic proportions in Scotland and Ireland; Edinburgh had 206 cases with 135 deaths, Glasgow 998 cases with 715 deaths, and Belfast 623 cases with 495 deaths.

The first large epidemic recorded in England occurred in 1915. It began late in 1914 amongst the Canadian Expeditionary Force (Reece 1916) quartered on Salisbury Plain, and rapidly spread, involving both the civil and the military population. The following figures are instructive (Rolleston 1919) (Table 121).

TABLE 121

ENGLAND AND WALES

	Cases.	Deaths.
1912	272	142
1913	304	163
1914	300	206
1915	2,343	1,521
1916	1,278	838
1917	1,385	906

During the war of 1914–18 the British Expeditionary Force in France was attacked with some severity; the French Army suffered less, and the German Army hardly at all.

New York suffered severely in 1904–05. In the latter year there were 2,755 cases with 2,026 deaths (Flexner 1907*a*). Extensive outbreaks were reported in France in 1909–10, and in Texas in 1912. More recently the disease has increased

in some countries. There was a particularly severe outbreak in the United States in 1928–30 (see Hedrich 1931). In England and Wales the incidence rose from 300 cases and 284 deaths in 1923, to 2,152 cases and 1,440 deaths in 1931.

The disease attacks particularly children and adolescents. Compton (1918) finds that the most susceptible age is 0 to 5 years; the susceptibility is slightly less from 5 to 10, distinctly less from 10 to 15, and for the remainder of life it remains fairly low—at about 1/5 of that of the 0 to 5 age period. In the Danzig epidemic of 1865, 93 per cent. were under 5 years of age. The disease is unusual, however, in the first 3 months of life. It is somewhat commoner amongst males, but this appears to be related more to increased opportunity for infection than to any special sex predisposition. Miners and soldiers are the two occupational classes on which the disease falls most heavily. The seasonal incidence is very marked, the greater proportion of cases occurring in winter and spring: during 1915 and 1916 in England, 77 per cent. of the cases were in the first six months of the year. Much has been written about weather conditions. A low temperature, a cold wind, and increased humidity of the atmosphere appear to be predisposing agents (Dopter 1921), but it seems probable that these act mainly by leading to overcrowding indoors. Fatigue exerts a powerful influence; it is the recruits, unaccustomed to the rigours of military life, who furnish the greatest number of cases in the Army. Dopter tells an impressive story of a party of recruits who made a long, fatiguing march to join their regiment at Versailles. On reaching their destination cerebrospinal fever broke out, and of a total strength of 153 men no fewer than 79 developed the disease.

II. Mode of Spread of the Disease.—The disease is endemic in large towns, from which, at least in the post-basic form, it rarely disappears. Every winter and spring a few fresh cases occur—sporadic cases affecting children, less frequently young adults—which appear at widely separated points having no apparent connection with each other. In villages the endemic state is practically unknown.

Every now and then an epidemic breaks out. As a rule it starts insidiously by the occurrence of one or two cases at intervals of a week or more; it then begins to advance by the formation of small multiple foci in families, schools, barracks, or gaols. Often it works itself out in one focus before passing on to another. Thus, at Strassburg (Dopter 1921) in 1840 the 7th line regiment was attacked in October; three months later it spread to the 69th regiment, of which two companies were quartered with a part of the 7th. In January the 29th line regiment and the 11th artillery were attacked; in February the 34th line regiment and the 1st artillery; and in March, 6 months after the commencement of the outbreak, it invaded the pontonniers.

In a town the cases are not aggregated together, but occur widely scattered, as if there were no causal relationship between them. Thus in Hamburg between 1880 and 1885 the 180 cases of cerebrospinal fever that occurred were distributed over 131 streets.

The spread of the epidemic is irregular and capricious; often groups, which from their situation appear certain to be attacked, escape, whereas others, situated remotely from the primary focus, are attacked. Similarly with regard to time; the successive outbreaks of an epidemic are irregular; there are paroxysms of intensity followed by remissions or intermissions. The decline of the epidemic takes place slowly, fewer and fewer foci remaining, till finally the endemic state is regained (Dopter 1921).

It is remarkable what a predilection the disease has for the military population; frequently garrisons have been attacked without a single case amongst the neighbouring civilians. Of 75 epidemics in France, collected by Hirsch (1886), 39 were confined to the troops. In Germany the miners of Westphalia and Silesia have suffered severely. In India it is the gaols that appear to have furnished most of the cases.

Two of the most striking features of a cerebrospinal fever epidemic are the low morbidity and the high case fatality. Dopter (1921) says that the morbidity varies between 0·01 and 0·3 per cent. of the population at risk, though occasionally it may rise higher. The case mortality is generally about 70 per cent.; it may be as low as 37 per cent., or as high as 90 per cent. During the years 1920–36 the average case-mortality rate in 22 cities of the United States was 51·2 per cent. (Walsh 1938).

Long before the discovery of the causal organism, it had been noticed that overcrowding favoured the development of the disease. The reason for this was not known. At a post-mortem examination of a case of cerebrospinal fever, Weigert noticed the presence of a purulent rhinitis, and on the basis of this observation von Strumpell suggested that the nasopharynx might serve as a portal of entry for the infective material. Weichselbaum (1887) in his original paper likewise mentioned that one of his cases had a purulent sinusitis, and expressed the opinion that the organisms might find their way to the meninges *via* the nose. While working with meningococci Kiefer (1896) developed a purulent right-sided rhinitis from which he cultivated Weichselbaum's diplococcus; he did not develop meningitis. During the next few years a number of workers claimed to have isolated the organism from the nose, not only of patients but of healthy contacts. Further work rendered it clear that not only were meningococci frequently present in the nasopharynx of patients suffering from cerebrospinal fever—about 60 per cent of cases in the first week of the disease—but also in the nasopharynx of contacts and even of non-contacts (Albrecht and Ghon 1901, von Lingelsheim 1905*a*, Kutscher 1906, Ostermann 1906). It became clear therefore that the meningococcus was able to vegetate in the nasopharynx—frequently without giving rise to meningitis—and that it could be transmitted from patients to healthy persons in the vicinity.

Certain observers, notably Dopter (1921), have come to the conclusion that the real disease to which the meningococcus gives rise is rhinopharyngitis; the meningitis which occasionally develops is to be regarded, not as the main disease, but as a complication, in much the same way as nephritis is often grafted on scarlatina or paralysis on diphtheria. Actual rhinopharyngitis does not appear to be a common sequel to meningococcal invasion, and it is probably more accurate to speak of this invasion as a "carrier epidemic." There is some evidence, however, that the meningococcus does not act as a pure commensal, because antibodies are sometimes formed as a result of its presence in the nasopharynx (Rake 1935*a*).

During the war of 1914–18 observations of much interest were made on the carrier rate. By swabbing large numbers of the military population in camps and depots, it was found that previous to an outbreak of cerebrospinal fever the proportion of carriers of the meningococcus increased steadily. The normal carrier rate amongst troops was recorded as 2–4 per cent., but preceding an epidemic, it rose till it reached 20–30 per cent. Soon after it had passed the 20 per cent. limit,

isolated cases of meningitis began to appear, and as the epidemic gained foothold the carrier rate likewise rose, sometimes to as high as 88 per cent.

Investigating the cause of this "warning rise" in the carrier rate, Glover (1920) was led to suspect a relationship between it and overcrowding in the sleeping huts. These huts were at the best poorly ventilated (Eagleton 1919–20), and during the stress of war the mobilization standard had been overstepped, so that beds, instead of being separated by 1 ft. 4 in., were practically touching each other. Glover (1920) noticed that the carriers in a given hut tended to be aggregated together; three Type II carriers were in adjacent beds, two Type I carriers, and so on. This pointed strongly to the direct transmission of the meningococcus from one man to another sleeping in the next bed. Finding that the spraying capacity during normal sleep was not more than about 3 ft., Glover tried the effect of spacing out the beds in the hope that the infection would be diminished. The results obtained seemed to be in accordance with expectation. The effect of the distance between the beds was not confined to the carrier rate. At Caterham depot, where there was severe overcrowding, an outbreak of cerebrospinal fever had occurred during each winter of the war, but subsequently to the adoption of the spacing-out policy in 1917–18, not a single case occurred.

Because of the far-reaching conclusions on the relation between overcrowding and the carrier rate, and the importance of detecting the "warning rise," Glover's work has received a great deal of attention.

In the Detroit epidemic of 1928–29, Norton and Baisley (1931) found no correlation between the degree of overcrowding in the home and the contact carrier rate. During the outbreak of 1931 at Aldershot, Armstrong and his colleagues (1931), from a study of the position of carriers in dormitory barrack rooms, were unable to obtain any evidence that infection occurred mainly at night. The carriers were scattered quite irregularly without any particular relation to the position of the beds. Our own nasopharyngeal surveys of the civilian population carried out before the second world war have shown us that the carrier rate in institutions may be as high as 20 per cent. and over, without any outbreak of cerebrospinal fever occurring. Rake (1934) has made the same observation. Perhaps the most striking figures, however, are afforded by Dudley and Brennan (1934). Working at the Chatham naval hospital, they found that between January, 1932, and March, 1933, there were 11 cases of cerebrospinal meningitis with a carrier rate of about 13 per cent. During the period March, 1933, to May, 1934, the carrier rate was 54 per cent., yet not a single case of meningitis occurred. During the same period at the Royal Naval Hospital, Portsmouth, there were 6 cases of meningitis with a carrier rate of only 5 per cent. Analysis of the distribution of carriers at Chatham showed no constant relationship between the density of the population and the carrier rate. The senior ratings with the most spacious sleeping accommodation had as high a carrier rate—60 per cent.—as the recruits with the worst sleeping quarters. Phair, Schoenbach and Root (1944) carried out a survey at an army camp in which cerebrospinal fever was prevalent. There were no cases, however, in the unit they examined. A group of 99 men was swabbed 28 times in 68 days; no fewer than 91 of these men were found on one or more occasions to be harbouring meningococci in the nasopharynx. The average carrier rate was 41 per cent.

These observations lend no support to the suggestion that there is a direct relationship between the carrier rate and the incidence of cerebrospinal fever.

That Glover's observations were correct, no one has questioned; but it may be doubted whether Glover was justified in attaching to them a general rather than a local significance. It is reasonable to suppose that overcrowding must increase the frequency of exchange of nasopharyngeal flora. Whether, however, this neces-

sarily results in a high carrier rate, and if so to what extent overcrowding favours the development of cerebrospinal meningitis, it is at present impossible to say. On the whole it seems probable that the extent of disease depends on the virulence of the invading organism as much as on its distribution. Judged by the mouse test, there is a considerable variation in the virulence of different strains of meningococci isolated from the human body (Cohen 1936). Group I strains, which are responsible for most epidemics, tend to be more antigenically homogeneous and more virulent than Group II strains, which predominate in inter-epidemic periods; and even among Group I strains differences in antigenic structure and in virulence can often be established (see Branham 1940*a*). We are forced, in fact, to much the same conclusion as Griffith in his study of scarlet fever (Chapter 66), namely that whether in an invaded susceptible community the disease becomes epidemic or not is determined largely by the characters of the infecting strain.

Routes of Infection.—There is now no doubt that the meningococcus gains access to the body *via* the nasopharynx. Several early observers claimed to have found the organism in various parts of the respiratory tract, and it was not long before its predilection for the nasopharynx was demonstrated. It is unusual to find it in the nose itself or in the saliva, where, as Gordon (see Report 1917) showed, the salivary streptococci exert an inimical action on its growth; it may be isolated from the throat, but the most important site is undoubtedly the nasopharynx. It is here that it multiplies, and sometimes sets up a rhinopharyngitis.

Whereas there is now general agreement that the nasopharynx is the portal of entry, it is still uncertain by what route the organism gains access to the meninges. There are two main views: the first, supported by Netter and Debré (1911), postulates the direct transmission of the organism from the nose to the meninges; the second, upheld by several workers in Germany, America and England (Westenhoeffer 1906, Elser and Huntoon 1909, Herrick 1918, Bacslack *et al.* 1918, Rolleston 1919), is that it is carried to the meninges by the blood stream. The available evidence is as follows.

(*a*) Direct Extension from the Nose.—The advocates of this view draw attention to the close relation existing between the subarachnoid space and the nose. Around each of the 15 to 18 branches of the olfactory nerve which pierce the cribriform plate of the ethmoid there is a sheath; between this sheath and the nerve itself there is a space which is in direct communication with the subarachnoid space.

Clark (1929) found that a solution of potassium ferrocyanide and iron ammonium citrate, instilled into a rabbit's nose, reached the surface of the brain within an hour. The distribution of the blue granules indicated that the path followed had been the perineural spaces of the olfactory nerves. Further work showed that material might pass from the nasal to the intracranial cavity by way of the blood vessels piercing the cribriform plate. Fine canaliculi, representing prolongations of the subarachnoid space into the olfactory mucosa, have also been described traversing the cribriform plate, homologous with similar prolongations found in the internal ear and around the optic nerve; but Clark was unable to satisfy himself that these canaliculi had any direct communication with the nasal lymphatic vessels. Methylene blue injected into the subarachnoid space beneath the frontal lobe of a dog colours not only the perineural prolongations, but also the lymphatic plexus of the nasal mucosa. The lymph flow appears to be centrifugal, whereas the flow of fluid (not lymph) in the perineural spaces may, as Clark's observations have shown, be centripetal, and thus carry particulate material from the nose to the brain (see also Findlay and Clarke 1935, Rake 1937*b*).

Another possibility is that the organisms may invade the nasal sinuses, set up a purulent

sinusitis, and reach the skull by the lymphatics, or by direct extension through the bone. Numerous observers have found a sinusitis in cases of cerebrospinal fever, particularly of the sphenoidal sinus. Embleton and Peters (1915) examined three patients who had died of the disease, and found in each a sphenoidal empyema due to the meningococcus. Against this view are the facts that, though not uncommon, sinusitis is by no means constant in cerebrospinal fever, and that in children, in whom the disease is commoner than in any other class, the sphenoidal sinus does not develop before the third year of life. Moreover it is possible that the involvement of the nasal sinuses may be secondary to the meningitis, the organism being carried by the natural flow of the lymph stream to the nose. Flexner (1907*b*) found in monkeys, injected intraspinally, that the organisms might find their way from the meninges to the nasopharynx.

As well as the transethmoidal and the sphenoidal routes, it has been suggested that spread may occur *via* the Eustachian tube to the middle ear and thence to the brain. This seems unlikely because of the rarity of otitis media in cerebrospinal fever, and of the lateness of its development when it does occur.

The advocates of direct lymphatic spread from the nose to the meninges object to the hæmatogenous route of infection on the ground that blood cultures are frequently sterile, that positive results are not more common at the beginning of the disease than during its later stages, and that the arthropathies and visceral lesions which sometimes appear as complications occur fairly late in the course of the disease, suggesting that the blood has become secondarily infected from the meninges; that this may occur in animals has been shown by Flexner (1907*b*), who found meningococci in the blood subsequent to intraspinal injection.

(*b*) THE HÆMATOGENOUS ROUTE.—Meningococci were found in the blood by Salomon in 1902; Jacobitz in 1905 reported two cases; Elser (1905–06) obtained 10 positive blood cultures out of 41—mostly in the first week of the disease; Dieudonné (1906) was successful in 4 cases out of 5. During the war of 1914–18 Baeslack (1918) and his co-workers cultivated the meningococcus 8 times from 22 cases, and Barber and Fleming (see Herrick 1918) 12 times from 15 consecutive cases. Moreover the organism has been cultivated from the petechial spots (Muir 1919), from the swollen periarticular lesions (Still 1898), from the endocardial vegetations, and from other situations clearly infected by the blood stream. Cases of chronic meningococcal septicæmia lasting for months, sometimes with ultimate recovery, are not uncommon (for references see Campbell 1943).

From these records it is clear that an infection of the blood is frequently present. It must be remembered that the meningococcus is not an easy organism to grow, and that if it is present in only small numbers the chances of obtaining it in blood culture are not great. With ordinary care, however, at least 25 per cent. of the blood cultures may be expected to be positive in the first week of the disease.

As well as the finding of the meningococci in the blood, other reasons have been advanced in favour of the hæmatogenous route. Clinical evidence shows that a meningococcal septicæmia may occur without the development of meningitis. Herrick (1918) states that 4 per cent. of patients with septicæmia fail subsequently to develop meningitis. Higher proportions than this have been reported by later workers (see, for example, Dickson *et al.* 1941, Daniels *et al.* 1943).

Westenhoeffer (1906), who at one time favoured the direct method of spread, but who later abandoned this view in favour of the hæmatogenous route, was struck by the presence in two cases of cerebrospinal fever of miliary abscesses in the myocardium and in the renal medulla; by the occurrence of cases of purulo-hæmorrhagic encephalitis; and by the finding of pus around the small arteries of the pia in a brain that otherwise appeared normal—all points suggestive of blood infection.

It is clear that the demonstration of meningococci in the blood cannot be regarded as evidence in favour of the hæmatogenous route, unless it can be shown that the blood is generally infected before the meninges; otherwise it is legitimate to argue that the blood-stream infection is secondary to the meningeal invasion. So far the number of cases

that have been studied with a view to determining the sequence of events is insufficient to enable any definite conclusions to be drawn.

Summing up, it may be said that the most probable sequence of events is as follows: The organisms reach the nasopharynx by air-borne infection; here they may set up a rhinopharyngitis, but more usually appear to give rise to no trouble. In some cases, however, the meningococci gain access to the meninges—whether by direct invasion or *via* the blood stream is not yet known. A meningococcal septicæmia in the absence of meningitis is not uncommon, and in a few instances lesions in other parts of the body develop, generally in the late stage of the disease, such as endocarditis, arthropathies, and renal abscesses. These are probably due to a secondary infection of the blood stream from the meninges.

Diagnosis.

In cerebrospinal meningitis the causative organism is always present in the cerebrospinal fluid; in a considerable proportion of cases it is also present in the nasopharynx. In this latter situation Elser (1905–06) found it in 6 out of 22 patients; Weichselbaum and Ghon (1905) in 18 out of 19 patients (not fully identified); Goodwin and von Sholly (1906) found it in 12 out of 22 patients in the first week of the disease, and in 5 out of 15 in the second week. Von Lingelsheim (1908) found it in 46 out of 49 patients in the early stage of the disease. Gordon (see Report 1920) states that it can always be found in the early stage of the disease, but that its isolation is more difficult later. For diagnostic purposes, therefore, it is essential to perform a lumbar puncture, and desirable to take a nasopharyngeal swab.

Examination of the Spinal Fluid.—The spinal fluid should be withdrawn and divided into three portions, which should be treated as follows:

(1) *Examination of centrifuged deposit for cells and organisms, and of the supernatant fluid for specific precipitinogens.* The fluid is centrifuged, the deposit stained by Gram, and examined microscopically for the presence of the oval Gram-negative diplococci. These vary greatly in numbers; sometimes they are present in every field, but in others they are not found until after a prolonged search; in this respect the picture is very different from that of septic meningitis, in which the pneumococci or streptococci are present in large numbers, and can be found without difficulty. In cerebrospinal meningitis the majority of the cocci are situated within the polymorphonuclear cells. Dissolved antigenic material, probably of polysaccharide type, may frequently be demonstrated in the spinal fluid. A few drops of the supernatant fluid are layered gently on to a similar quantity of monovalent or polyvalent antimeningococcal precipitating serum in a Dreyer's tube. Usually a fine white opaque disc of precipitation becomes apparent in a few minutes. If it does not, the tube is incubated in a water-bath for 1–2 hours at 37° C. Both Rake (1933*b*) and Maegraith (1935) have obtained very satisfactory results with this method (see also Alexander and Rake 1937). In Maegraith's series of 120 cases, a positive reaction occurred on 116, while of 180 control fluids, all but 2 were negative. No other method affords such a rapid diagnosis.

(2) *Immediate plating.* As soon as the spinal fluid is withdrawn, it should be streaked in quantities of 1 ml. over 2 or 3 plates of ascitic agar or Gordon's trypagar (Gordon *et al.* 1916). These plates should be incubated immediately, or if this is impossible, they must be transmitted to the laboratory in a warm container. The

less their temperature falls below 37° C., the more likely is growth to occur, and the shorter is its lag period. Growth is often greatly favoured by the addition of 5–10 per cent. CO_2 to the atmosphere. In favourable circumstances colonies should be formed within 18 hours. These are examined with a hand lens, and films are made in the usual manner. A growth of Gram-negative diplococci having been obtained, it is important to ascertain their serological type. If there is a heavy growth on the plates, this may be suspended in a small quantity of saline, heated to 65° C. for 1 hour, standardized to 500 million per ml., and tested against the type sera; incubation should be performed by the partial immersion method in a water-bath for 4 hours at 50° C. A more rapid result is said to be obtained by the use of specific antisera prepared by the injection of chickens; readings can be made in about 5 minutes (Phair, Smith and Root 1943). If the growth is insufficient for a tube test, or if an immediate answer is required, slide agglutination may be used instead (Bell 1920*b*), but the results should always be checked by the tube method. If there is insufficient growth on the primary plates to allow of either method of agglutination, subcultures must be made on to fresh plates, and the growth examined after 24 hours. The fact must not be forgotten that one variety of organism found in meningitis, *N. flavescens*, may produce a golden-yellow pigment (Branham 1930).

It must be pointed out that not all strains are readily agglutinable by the available type sera. Strains from sporadic cases are usually less readily agglutinated than those isolated during an epidemic. Much depends upon the strains selected for the preparation of the agglutinating sera (see Griffith 1917, Scott 1917, Bell 1920*a*, Murray 1929, Branham 1932, Rake 1933*a*).

It is important to test the fermentation reactions of the organisms isolated. For this purpose colonies should be inoculated into glucose, maltose, and sucrose peptone water, containing 10 per cent. of ascitic fluid or blood serum. The meningococcus produces acid in glucose and maltose, but not in sucrose; *N. flavescens* is without action on any of the sugars. The final identification of any strain must be made on the basis of morphological, cultural, fermentation, and serological reactions.

(3) *Incubation of the spinal fluid with subsequent subcultivation.* It is always wise to incubate 5 ml. of the fluid at 37° C. in a sterile tube, in case direct plating proves negative. Sometimes the organisms develop in the fluid itself, when they fail to do so in cultures made at the time of lumbar puncture. As soon as turbidity appears, the fluid should be subcultured on to suitable media, and the resulting colonies identified in the usual way.

The frequency with which positive cultures are to be expected depends partly on the stage of the disease and partly on the carefulness of one's technique. Flack (see Report 1917), in an examination of 55 specimens of cerebrospinal fluid, obtained the following results:

	No.	Culture Positive. Per cent.
In clear fluids	15	33
In slightly turbid fluids . .	13	61
In turbid fluids	27	96

In the early stages of the disease before the spinal fluid is purulent, the proportion of positive cultures is much lower than in the later stages. In chronic

cases the fluid, after being frankly purulent, may clear again, and the organisms become increasingly difficult to isolate. Ultimately they disappear and can no longer be demonstrated.

Cultivation from the Nasopharynx.—At the time of lumbar puncture a nasopharyngeal swab should be taken. There are two advantages in this practice. First, a positive culture may be obtained from the nasopharynx, especially in the early stages of the disease, when the spinal culture is negative. Second, the nasopharyngeal swab may yield a heavier growth than the spinal plate, and so enable the serological typing of the organisms to be carried out more rapidly. Since the nasopharyngeal and spinal strains are almost invariably of the same type, there is little danger of error in this procedure.

For examining the nasopharynx, West's swab may be used; this consists of a wire curved at one end, fitted with a pledget of cotton-wool, and enclosed in a glass tube. As soon as the swab has been taken it should be streaked over a plate of ascitic agar or Gordon's trypagar; if the latter medium is used, it is advisable to smear 2 or 3 drops of fresh blood over the surface. The plates must be incubated immediately, or kept in a warm container. The following day they are examined for the typical lenticular colonies of meningococci. The further examination of these colonies should be performed in the same way as that of the spinal colonies. If the swab cannot be inoculated at once on to a suitable medium, it should be stored in a tube with a little blood agar at the bottom with the swab touching the agar (Downie 1940). Whatever type of swab is used, it is important to avoid its becoming contaminated with saliva, otherwise the meningococci may be overgrown by salivary streptococci.

The serological typing of meningococci isolated from the nasopharynx often presents difficulties, particularly during inter-epidemic periods (see Chapter 23). During the course of an epidemic, the proportion of agglutinable to inagglutinable strains may rise considerably, depending probably upon the selection of a virulent epidemic strain of well-defined antigenic specificity (Report 1920). The higher the carrier rate is, the more likely are the organisms to be agglutinated.

Examination of the Blood.—(1) *Cultivation.*—An attempt may be made to cultivate the meningococcus from the blood, although this procedure is not adopted as a routine. For this purpose 10 ml. of blood should be withdrawn from the median basilic vein, and distributed in 1 ml. quantities over a series of 10 ascitic agar plates; or two flasks containing 150 ml. of 33 per cent. ascitic fluid broth may each be seeded with 5 ml. blood. Positive results may be expected in 25 per cent. of cases examined during the first week of the disease.

(2) *Demonstration of Antibodies.*—The blood may be examined for specific antibodies in special cases; but the diagnostic value of such tests is very doubtful, and they must never be regarded as a substitute for the direct cultural examination.

It has been found that agglutinins are frequently present in the blood serum of patients suffering from cerebrospinal fever (Dieudonné 1906, MacGregor 1910). Von Lingelsheim (1908) examined the sera of 593 patients. On the 1st day of disease 24·1 per cent. gave a positive agglutination reaction; from the 6th to the 20th day, 52·7 per cent.; after the 21st day, only 26·7 per cent. of positive reactions were obtained. The titre varies considerably. Bettencourt and França (1904) found that it was usually 1–10 to 1–50; occasionally titres of 1–200 or even 1–1000 were met with. Von Lingelsheim (1905*a*) gives the following figures:

146	agglutinated at	1-10
86	„ „	1-25
30	„ „	1-50
8	„ „	1-100
1	„ „	1-200
149	failed to agglutinate at	1-10

Gates (1918) found agglutinins almost constantly in the blood of carriers of 4 to 16 weeks' standing; the titre was generally 1-16 to 1-32.

It is seen that agglutinins are not present in more than 50 per cent. of cases as a rule. MacGregor (1910) found that when marked toxæmia was present, there were generally no agglutinins; likewise in chronic and in abortive cases. They were present in greatest quantities in cases with an acute onset and fairly high fever. Other observers (Elser and Huntoon 1909) have found the agglutination reaction to be too irregular for diagnosis.

Houston and Rankin (1907), studying the opsonic content of the serum of patients suffering from cerebrospinal fever, found that it was raised in 25 per cent. of patients on the 2nd day of the disease, in 60 per cent. on the 5th day, and in 96·1 per cent. after the 6th day. They stated that the opsonin test was of great value in the diagnosis of suspected cases. MacGregor (1910) found opsonins present in greatest quantity during the 2nd and 3rd weeks of the disease; like agglutinins, they are said to be most abundant in cases with acute onset. Cruickshank (1941) states that circulating antibodies can often be demonstrated in the second week of the disease, and perhaps even earlier in cases of meningococcal septicæmia.

Examination of Petechial Skin Lesions.

The presence of meningococci in petechial spots was demonstrated by Muir (1919), using the cultural method. In a considerable proportion of cases the organisms can be found microscopically in smears. McLean and Caffey (1931) were successful by this means in 14 out of 18 cases in children, and Tompkins (1943) in the Army in 39 out of 48 cases. The petechial spot—macules are unsatisfactory—should be punctured with a Hagedorn needle, while the surrounding skin is pinched so as to prevent the access of capillary blood to the lesion. Small drops of blood should be smeared on to a slide and stained with Giemsa. Typical intracellular diplococci with the adjacent surfaces flattened can be detected in this way, and confirmed by a Gram stain. They can often be found when blood culture is negative.

Post-mortem Examination.—In the examination of bodies at autopsy, it is important to take cultures within 12 hours of death if possible; the meningococcus dies out rapidly, and though it may be found microscopically in smears from the meninges, cultures often prove to be sterile. For cultural purposes, it is best to use the pus on the meninges; if there is no pus visible in this situation, the interior of the lateral ventricles should be carefully examined; occasionally some may be found in the posterior horn. In cases of doubt the spinal cord should be removed, and a careful search made, especially of the posterior aspect.

Prophylaxis.

We have seen that the meningococcus lives as a parasite in the nasopharynx. Under normal conditions it will be found in a certain proportion of apparently healthy persons. Mathews and Herrold (1918) found it in 4·4 per cent. of non-contacts in a military camp during an epidemic; Ponder (1918) in 13 per cent. of non-contact civilians in a non-epidemic period; Scott (1916) in 22 per cent. of non-contact hospital out-patients; Nabarro (1917) in 6·3 per cent. of children attending a hospital. As a conservative estimate we may say that in non-epidemic

times the meningococcus is present in 2–8 per cent. of healthy civilians; the actual proportion will depend to a certain extent on the time of year.

We have also seen that the meningococcus is transmitted from carriers to normal persons around them. The carrier is clearly of great importance in the continued propagation of infective material. It is of interest, therefore, to ascertain how long the organism survives in the nasopharynx. Gordon and Flack (see Report 1917) and Mathews and Herrold (1918) observed that there are two classes of carriers; in the one class, only a few colonies of meningococci are found on the plates streaked from a nasopharyngeal swab; in the other class, there is an almost pure growth of meningococci. The former class clears up rapidly; the latter may carry the organism for months.

Flack (see Report 1917) gives the following table relative to the length of time that carriers may remain infective (Table 122).

TABLE 122

	Contact Carriers.	Per cent.	Non-contact Carriers.	Per cent.
Under 2 weeks	20	16·1	12	19·7
From 2–4 „	45	36·3	32	52·5
„ 4–6 „	26	21·0	5	8·2
„ 6–8 „	14	11·3	2	3·3
„ 8–10 „	4	3·2	7	11·5
„ 10–12 „	5	4·0	—	—
Over 12 „	10	8·1	3	4·9

Thus the percentage of carriers of more than 4 weeks' duration was 47·6 per cent. in the case of 124 true contacts, and 27·9 per cent. in the case of 61 non-contact carriers. A comparison of the persistence of different serological types of the meningococcus in the nasopharynx failed to reveal any marked variation between them. Norton and Baisley (1931) in the civilian epidemic at Detroit found that 72·4 per cent. of contact carriers became negative within a fortnight. Not all carriers, however, clear up. During inter-epidemic times some persons carry the meningococcus for months and even years; the organism, in fact, takes its place in the normal nasopharyngeal flora. Convalescent cases of cerebrospinal fever, it may be noted, harbour the meningococcus in their nasopharynx for a long time; Embleton and Steven (1919) found the average length of carriage to be about 6 months.

Apart from droplet infection by carriers, it is possible that indirect infection may play a small part in the spread of the disease. Dopter (1921) considers that this may occur occasionally by handkerchiefs, bedding, clothes, and drinking vessels contaminated with nasal mucus. It is difficult to say how frequent such indirect infection may be, but in view of the extreme susceptibility of the meningococcus to drying and to cold, we may regard it as negligible. Neither in barracks nor in civilian households is this method of infection to be compared with the direct infection by nasal mucus sprayed from the carrier.

In connection with the prevention of cerebrospinal fever much has been written on the advisability of detecting and isolating carriers. Hachtel and Hayward (1911) were able to eliminate the disease from an orphan children's home by isolating the carriers, treating them with topical applications of anti-

whether in civilian life the manifest advantages of the method are outweighed or not by its disadvantages.

Prophylactic Vaccination.—The value of prophylactic vaccination is difficult to ascertain. No definite conclusions can be drawn from the earlier attempts (see Sophian and Black 1912, Greenwood 1916, Gates 1918). More recently Zrůnek and Feierabend (1931) endeavoured to immunize the Czechoslovak army. Soldiers whose names began with A-M were vaccinated, while those with names beginning with N-Z were left as controls. Three doses—2,000, 4,000, and 4,000 million—of a vaccine prepared from recently isolated local strains killed at 60° C. for 1 hour were given. The inoculations were made in December, just prior to the usual seasonal outbreak of the disease. Of 21,280 subjects vaccinated in 1926 and 1927, 5 developed meningitis and 3 died. Of 19,684 controls, 6 developed meningitis and 3 died. Unfortunately for the success of the experiment, meningitis was much less prevalent in 1927 and 1928 than in previous years. The evidence, so far as it goes, does not, however, suggest that vaccination confers a high degree of immunity to the disease. Riding and Corkill (1932) carried out a large series of vaccinations on natives in the Northern Sudan. One group of 10,691 men received a polyvalent meningococcal vaccine, another group of 10,451 received T.A.B. vaccine, while 245,058 served as controls. No significant protection was afforded by either of the vaccines.

Serum Treatment.—The results of this treatment recorded by different observers are somewhat confusing, and their significance is by no means easy to assess.

Jochmann (1906) was the first to introduce this method of treatment. He prepared a polyvalent serum by injecting horses, first with dead then with living cultures, and found that it would protect mice against 4-6 M.L.D., provided it was given 24 hours beforehand in a dose of 0·5 ml. subcutaneously or intraperitoneally. He showed that the serum contained agglutinins, bactericidins and bacteriotropins. He then employed his serum for the treatment of human patients; he gave it in 20 ml. doses subcutaneously or intrathecally; 12 out of the 17 cases treated recovered.

Kraus and Doerr (1908) prepared a serum by the injection of goats and horses with broth filtrates and endotoxin; the resulting product was able to neutralize the endotoxin *in vitro*, and to protect guinea-pigs against subsequent injection with a lethal dose of toxin.

In spite of the work done by the early observers, it is to Flexner (1907*c*, 1912, 1913, Flexner and Jobling 1908*a*, *b*) more than to any other, that we owe our present knowledge of the value of serum treatment. In 1907 he (1907*c*) prepared sera from the goat, horse, rabbit and guinea-pig. He found that in monkeys the simultaneous injection of serum and of meningococci intraspinally was not followed by the development of severe symptoms, although the organisms persisted for a time in the spinal fluid; further, by injecting serum 6 hours after the infecting dose, the monkey—already seriously ill—could be saved from apparently impending death. Encouraged by these results he proceeded to try the serum on human cases. Together with Jobling (Flexner and Jobling 1908*a*) he prepared his serum by subcutaneous injection into horses at weekly intervals of living cocci and autolysate alternately; an attempt to standardize it was made by titrating it according to the complement-fixation test, and against the autolysate in guinea-pigs, but neither method was found to be quantitatively reliable. The serum was distributed from the Rockefeller Institute in New York to all parts of the world; reports were

received from the practitioners using it, and in 1913 these were finally collected and analysed (Flexner 1913).

Excluding fulminant cases dying within 24 hours of serum injection, cases with mixed infection of the meninges, cases of intercurrent infections, and hopelessly chronic cases, of a total of 1,294 patients treated by their serum, 894 recovered and 400 died, giving a mortality of 30·9 per cent. This mortality must be compared with that of untreated cases, *from which, however, similar exclusions had not been made*. At this time the mortality of cerebrospinal fever in Great Britain was from 70–80 per cent., in Germany 42–67 per cent., in France 75 per cent., in Belgium 77·7 per cent., and in Italy 55·6 per cent., in Palestine over 80 per cent., in Greece 58·6 per cent., and in the Transvaal 74 per cent. From these figures it may be concluded that the mortality of non-serum-treated cases was about 70 per cent. In 1,211 treated cases the reports were sufficiently full to permit an analysis of the effect of the time of administration (Table 123).

TABLE 123

Time of Injection.	No. of Cases.	Recovered.	Died.	Per cent. Recovered.	Per cent. Died.
1–3 days	199	163	36	81·9	18·1
4th–7th day . . .	346	252	94	72·8	27·2
Later than 7th day .	666	423	243	63·5	36·5
Totals	1,211	838	373	69·2	30·8

This table brings out the beneficial effect of treatment of cases admitted early to hospital. The mortality of cases in which serum treatment was commenced before the 4th day was only half that of cases not treated till the 2nd week or later.

Another interesting point is the fatality of serum-treated patients at different ages (Table 124).

TABLE 124

MORTALITY ACCORDING TO AGE IN SERUM-TREATED PATIENTS.

Age.	No. of Cases.	Recovered.	Died.	Per cent. Recovered.	Per cent. Died.
Under 1 year . .	129	65	64	50·4	49·6
1–2 years .	87	60	27	69·0	31·0
2–5 „ .	194	139	55	71·6	28·4
5–10 „ .	218	185	33	84·9	15·1
10–20 „ .	360	254	106	70·6	29·4
Over 20 „ .	288	180	108	62·5	37·5
Age not given . .	18	11	7	61·1	38·9
Totals	1,294	894	400	69·1	30·9

From this table it is seen that infants under 1 year of age have the highest mortality, and children between 5 and 10 years the lowest. The high infantile mortality may be largely due to the difficulty of diagnosis, so that serum treatment is often not commenced till after the disease has lasted a week, and partly also to the technical difficulties inherent in lumbar puncture and in serum administration in the very young.

A further study of the records seemed to show that in cases favourably influenced by serum, recovery occurred fairly rapidly; not infrequently the temperature, which in untreated patients generally falls by lysis, fell by crisis. This was especially noticeable in cases treated early.

Following the injection of serum, the blood leucocytes fell from a figure of 20,000–40,000 to normal within a week or so; the spinal fluid became progressively clearer, the pus cells grew fewer, and the meningococci disappeared. On the other hand, in cases not influenced by serum the leucocytosis remained high; the spinal fluid showed an increase in the turbidity, and in the number of pus cells and living meningococci.

In cases untreated by serum which did not prove rapidly fatal, the disease often passed into a chronic stage lasting for months; or if recovery occurred, it was apt to be followed by a relapse. In serum-treated cases, however, it was found that the patients either died or recovered completely. Only very rarely did the disease become chronic. Relapses were likewise uncommon; they occurred in only 8 per cent. of cases, and frequently yielded to further serum treatment.

Another favourable indication of the value of serum was the diminution in the proportion of patients who developed complications. These complications—irido-choroiditis, panophthalmitis, deafness due to internal ear disease, pleurisy, pericarditis, endocarditis, arthropathies, insanity and others—are not uncommon in patients who recover naturally. In serum-treated patients such complications were few; the only one which occurred with any frequency was deafness; this was doubtless due to the early spread of the disease to the internal ear; the sooner serum treatment was begun, the less likely was this spread to occur.

Summing up Flexner's results, it would appear that by serum treatment the mortality was considerably reduced, the frequency of relapses diminished, the incidence of chronic cases abolished, and the proportion and the severity of the complications lowered very considerably.

There are numerous records by other workers of cases treated by serum with results essentially similar to those obtained by Flexner (see Netter and Debré 1911, Adshead 1918, Rolleston 1918, Hines (Report 1920), Dopter 1921, Wadsworth 1931, Sturdee and Scott 1933, Banks 1938). Univalent sera have, on the whole, been more effective than multivalent sera.

Before judging the value of serum treatment there are certain factors of importance to be considered.

The first concerns the **mode of preparation** of the serum. This has varied in different countries.

Flexner and Jobling (1908*a*) used subcutaneous injection of living diplococci and of an autolysate alternately; this method, though effective, requires considerable time—10 months or more—before the serum is sufficiently potent for use. Amoss and Wollstein (1916) described a more rapid procedure, yielding a multivalent serum of high titre in 8 to 12 weeks; the essential feature of their method consists in giving 3 successive intravenous injections of living meningococci at daily intervals, followed by a rest of 8 days, before the administration of a fresh series. Numerous strains of different types are included. A. S. Griffith (see Report 1920) followed this method in his preparation of sera for the Medical Research Council, with the exception that he used strains of only one type for each horse; the resulting sera were therefore univalent. It is important in selecting the cultures to be used for immunization to choose strains which are representative of those present during an epidemic. F. Griffith (1917) advises the use of the highly complex strains which are found more commonly at the height of an epidemic than at any other

period. In the preparation of a multivalent serum Wadsworth and Kirkbride (1926) favour the use of 4–6 carefully selected strains rather than of larger numbers. On the ground that meningococci produce a soluble toxin (see Ferry, Norton, and Steele 1931), Ferry (1932, 1934) recommends antitoxic sera prepared by injecting horses with the type-specific toxins. The evidence, in his opinion, suggests that antitoxic are of more importance therapeutically than antibacterial sera. Maegraith (1938), however, was unable to confirm this. The antibody in native sera may be concentrated by suitable fractionation (Neal *et al.* 1928, Murdick and Cohen 1933, 1935), but whether such sera are more efficacious in practice than ordinary sera still remains to be seen.

The next factor to be considered is the **titration** of the serum. As yet there is no laboratory method by which the therapeutic power may be correctly gauged.

Flexner and Jobling (1908*a*) relied partly on the complement-fixation test, and also on titration against autolysate in guinea-pigs. Nicolle, Debains and Jouan (1918) used the complement-fixation test alone; Jobling (1909) the opsonin content; Amoss and Wollstein (1916) the agglutinin content; Hitchens and Robinson (1916) a mouse-protection test; Gordon (see Report 1920) the anti-endotoxic value; and de la Rivière and Roux (1925) a precipitin test. As the results given by these different methods vary considerably, it is clear that they cannot all be satisfactory. Experience during more recent years has similarly failed to reveal any substantial agreement between different workers on the best method of titration. Wadsworth (1931) favours agglutination, Zozaya (1932) and Mishulow (1935) precipitation, Ferry, Norton and Steele (1931) and Konowa and Nikolsky (1934) toxin-neutralization, Sickles (1933) a neutralization test based on skin reactivity, and Zdrowdowsky and Voronine (1932), Branham (1935), and Rake (1935*b*) some form of protection test. On the whole, opinion is now crystallizing in favour of the intraperitoneal mouse protection test (Mishulow *et al.* 1936, Rake 1937*a*, Branham *et al.* 1938, Branham and Pittman 1940). There seems to be little relation between the agglutinin and the protective titres in any given serum, but according to Rake the protective antibody content is fairly well correlated with the amount of immune nitrogen precipitable by type-specific polysaccharide (see also Pittman *et al.* 1938). Standardization in terms of some significant unit is clearly desirable before liberation of a serum from the laboratory; otherwise normal sera, or sera containing only a minimal antibody content, may be distributed by certain agencies. What this unit is to be still awaits international agreement. (For general discussion, see Murray 1929.)

Another factor is the **dose** of serum to be injected.

In an acute case in an adult it is advisable to inject 20–30 ml. of serum to start with, after having drawn off rather more than an equivalent amount of cerebrospinal fluid. The quantity of fluid which should be withdrawn varies from case to case; if it is under great pressure, it may be allowed to run until it is coming at the rate of 1 drop per second. Sometimes 120 ml. may flow away before this rate is reached. But the patient must be watched very carefully during the process, and if any untoward signs are observed, the flow must be stopped immediately. Generally it is better, if the fluid is under great pressure, to withdraw about 50 ml., and to repeat the lumbar puncture 12 hours later. The serum should be injected at least once a day for the first 4 or 5 days; sometimes it is desirable to give it twice daily. As in diphtheria, the amount given must be judged by the clinical condition of the patient. In fulminant cases, for example, greater risks may be taken than in less acute cases.

The **route** of injection is also of importance. Since meningococci are frequently present in the blood stream it has been advised that the serum should be given not only intrathecally but also intravenously. Herrick (1918) treated 137 cases by spinal injections with a mortality of 34·3 per cent.; and 128 cases by combined spinal and intravenous injections with a mortality of only 14·8 per cent. Further, he found that the greater

the amount of serum injected, the lower was the mortality. He advises giving about 100 ml. intrathecally and 400–600 ml. intravenously. Fewer complications were encountered when the combined method was employed. In the pure meningococcal septicæmias without coincident meningitis, the serum should always be administered intravenously. In chronic cases, with blockage of the spinal canal, serum should be injected sub-occipitally into the cisterna magna (see Report 1931). Very favourable results are said to follow this method (Cassoute *et al.* 1933, Alliezand and Carbonel 1933, Sepet *et al.* 1933, Robertson 1935). In cases that develop arthropathies, serum may be advantageously injected into the inflamed joints.

These are the main factors influencing serum treatment—the mode of preparation, the method of titration, the amount injected, and the route of injection. Taking all these into consideration, the results obtained must be considered as relatively favourable. It would seem that in cases adequately treated with a good univalent serum, Type I in particular, the mortality may be reduced by over 50 per cent. The adjustments that have been made in arriving at certain of these figures are, however, clearly unjustifiable, and so far as we know serum has never been tested under statistically satisfactory conditions. To do this, it would be necessary to give the serum to alternate cases in an epidemic, and to treat both series of cases in other respects in identically the same way. This would mean performing a lumbar puncture on the control cases with the same frequency as on the serum-treated cases, and replacing the fluid withdrawn by normal serum. The effect of lumbar puncture alone is probably of value. Until such a series is tried, we shall not be able to form a just opinion of the specific activity of the serum itself. The facts, however, that the earlier the serum is administered, the better are the results; that certain batches of sera appear to be more efficacious than others; and that the combined intrathecal and intravenous method gives a lower mortality than the intrathecal method alone, seem to us to point in favour of the therapeutic value of the serum itself.

Chemotherapy.—The introduction of the sulphonamide group of drugs has greatly influenced the case fatality of the disease. On this, all observers are agreed. Numerous figures might be quoted to indicate the reduction in mortality that has followed the use of these drugs, but so many factors—choice of drug, dosage, route, age of patient, and stage, nature and severity of disease—affect the result that comparisons, unless given in considerable detail, are apt to be misleading. As an example, however, it may be stated that Harries (1942) treated 500 consecutive cases of all ages in the Cardiff city isolation hospital with a gross mortality rate of 8·6 per cent.; this figure included every case admitted, even those that died within a few minutes of reaching hospital. Sulphapyridine was used for 471 of the cases, sulphathiazole for the remaining 29. In his series, as in those recorded by other observers (see Beeson and Westerman 1943, Report 1944), the case mortality was lowest in young adults and highest in infants and old people. Experience with different drugs has varied. Sulphapyridine has been widely used, but there is evidence that sulphanilamide may be better (Report 1944). Sulphathiazole has been recommended by Banks (1941) and others, and in the United States sulphadiazine has been favoured. It is important to institute therapy at the earliest possible moment, but experience suggests that the large doses recommended by Banks (1939) are not always necessary.

Whether the combined administration of sulphonamides and serum offers any advantage over sulphonamide treatment alone is still undecided. Both Amies

(1940) and Branham (1940*b*) have reported favourably on combined treatment in experimental infections of mice, but clinical reports (see Banks 1939, Beeson and Westerman 1943, Report 1944) do not seem to bear this out. On the other hand it must be remembered that exact comparisons on patients are difficult to make, and that unless all cases are treated by the same observer using the alternate method of administration, the results are apt to be misleading. There is certainly no convincing evidence that the serum does harm, and there is reason to believe that it may be of benefit in infections with drug-resistant strains. If it is given along with sulphonamide, it should be injected intravenously rather than intrathecally. More recently, penicillin has been suggested for the treatment of cerebrospinal meningitis, but it is too early to assess its value in this respect. According to Rosenberg and Sylvester (1944), intravenous or intramuscular injection of a suitable dose results in the appearance of penicillin in adequate concentration in the cerebrospinal fluid, suggesting that intrathecal administration is unnecessary.

Pneumococcal Meningitis

This form of meningitis occurs as a complication of lobar or broncho-pneumonia, and is commoner in children than in adults. Not infrequently in children it is secondary to middle-ear disease. Sometimes a primary pneumococcal meningitis is seen. The pneumococci are present in large numbers in the cerebrospinal fluid, which is turbid and full of pus cells; the cocci may be recognized by their appearance as Gram-positive, lanceolate, capsulated, and mostly extra-cellular diplococci. Cultures are best taken on to blood agar or ascitic agar. The type of coccus may be determined in the usual way. In untreated cases the disease is almost uniformly fatal. Serum treatment with univalent type serum may be tried, preferably by the combined intravenous and intrathecal routes, but far more favourable results are to be expected from the administration of the sulphonamides (see Coleman 1940).

Streptococcal Meningitis

Streptococcal meningitis is most frequently due to extension from middle-ear disease, especially in children, and to perforated wounds of the skull. The cerebrospinal fluid is turbid, and contains large numbers of pus cells and of streptococci, which are usually of the β-hæmolytic type. Before the sulphonamide era recovery was regarded as exceptional, though Watson-Williams (1938) points out that early mastoidectomy, combined with repeated lumbar puncture and the administration of antistreptococcal serum, may result in a substantial proportion of recoveries. In practice, sulphonamide therapy should be instituted as early as possible, along with appropriate surgical measures.

Tuberculous Meningitis

Tuberculous meningitis is probably always secondary to a tuberculous focus in some other part of the body, but, as it is often difficult to determine the site of the primary lesion, even at autopsy, the disease not infrequently appears to be primary. Osler (Osler and McCrae 1920) states that it may occur during the 1st year of life, but is commonest between the 2nd and 5th years; it is, however, not uncommon in adults, particularly as a terminal infection in pulmonary tuberculosis. The laboratory diagnosis is often difficult. Lumbar puncture reveals a fluid which may be limpid, and may contain only mononuclear cells. The protein

content is, however, always raised, while both the sugar and the chloride content are much diminished. The number of cells is generally increased, the most usual count being between 50 and 400 per c.mm. In most cases these cells consist chiefly of lymphocytes, but these are almost always associated with numerous polymorphonuclear and often with some plasma cells. If the fluid is allowed to stand at room temperature, a clot not infrequently forms; this should be spread on a slide, and stained by Ziehl-Neelsen. If no clot forms, the fluid should be centrifuged at high speed for half an hour, the deposit spread on albuminized slides, or fixed with methyl alcohol, and stained with Ziehl-Neelsen. It is often only after prolonged search that a group of tubercle bacilli is found. As well as being examined microscopically, the clot or centrifuged deposit should be injected intramuscularly into a guinea-pig, which is killed after 4–8 weeks (see Chapter 59). Sometimes it is possible to culture the organisms when none are visible microscopically; for this purpose the deposit should be distributed over 3 or 4 Dorset egg tubes, and incubated for a month. The bovine type is responsible for about 23 per cent. of cases in this country.

The disease is uniformly fatal. A few cases have been reported which have recovered, but it is possible that they are explicable by an error in diagnosis.

Influenzal Meningitis

This form is met with sporadically; it is not commoner during an influenza epidemic. Most cases appear to be primary. It was first described by Slawyk (1899), and has since been recognized by a number of workers (see particularly Cohen 1909, Ritchie 1910, Henry 1912, Rivers 1922, Schnyder 1925, Koch and Krämer 1931, Eddy 1933, Neal *et al.* 1934, Dahr 1934). Neal and her colleagues met with 111 cases of this type among a total of 2,727 cases of meningitis. The incidence is highest in childhood. It is rare under 2 months or over 6 years of age (Fothergill and Wright 1933). About 60–80 per cent. of cases occur during the first 2 years of life. The disease closely simulates cerebrospinal meningitis. It usually lasts 10–20 days, and has a case mortality rate of about 95 per cent. The cerebrospinal fluid is under pressure, is cloudy or purulent, has a high cell count, especially of polymorphs, and shows a variable increase in protein and often a marked decrease in sugar. Gram-negative bacilli are present with the typical Pfeiffer morphology, but sometimes accompanied by long thread forms. In the early stage of the disease they are intracellular, but later they become free in the fluid. On suitable media they can be cultivated from the spinal fluid, and often from the blood, and from the purulent joint exudates that occur not infrequently as a complication of this disease. In their nutritional requirements and their indole production they resemble *H. influenzæ*, but they differ from this organism in being capsulated, in producing distinctive colonies on Levinthal's medium, in forming a type-specific polysaccharide, and in being considerably more virulent for laboratory animals; they correspond to Pittman's Type b (see Pittman 1931, Mulder 1939). Occasional strains, however, of the respiratory type are encountered (Gordon, Woodcock and Zinnemann 1944). Inoculated intraperitoneally into rabbits, guinea-pigs, rats, and mice, they often give rise to a fatal septicæmia and to purulent effusions in the serous cavities. Treatment with sulphonamides is often successful (see Davies 1943, Archer and Singer 1943). Alexander and Leidy (1943), on the basis of mouse experiments, recommend sulphadiazine given alone or in combination with antiserum (see also Boisvert *et al.* 1944).

Other Forms of Meningitis

Occasionally meningitis may be caused by organisms, such as *B. aerogenes*, *Bact. coli*, Friedländer's bacillus (Gordon and Norton 1930), *Salm. typhi*, *Salm. enteritidis* (Stevenson and Wills 1933), *Salm. paratyphi B* (Patterson 1942), *Pf. mallei*, *Br. suis* (Hartley *et al.* 1934, Hansmann and Schencken 1932), the gonococcus (Strumia and Kohlhas 1933, Branham *et al.* 1938), *Diplococcus mucosus* (Cowan 1938, Bray and Cruickshank 1943), *Actinomyces bovis* (Henry 1919), and *Leptospira icterohæmorrhagiæ* (Marie and Gabriel 1935, Mollaret and Erber 1937).

For meningitis due to filtrable viruses, see Rivers and Scott (1935) and pp. 1932–1935.

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CHAPTER 65

GONORRHŒA

HISTORY.—That gonorrhœa has a history stretching back into antiquity we know, but we are entirely ignorant of the time and place of its first appearance. There is little to suggest that the disease has altered in its nature during the passage of time; it appears to run its course now in the same way as it has done for hundreds of years. It therefore differs from many diseases in which the clinical picture alters with the centuries. The explanation of this may lie in the absence of both natural and acquired immunity in man.

The causal agent, the gonococcus, was discovered by Neisser in 1879, who found it in 35 cases of gonorrhœa which had lasted for varying lengths of time, from 3 days to 13 weeks. He failed to find it in non-gonorrhœal pus, such as that accompanying chancres, buboes, etc., or in simple vaginal discharges; he demonstrated its presence in 7 cases of ophthalmia neonatorum, and in 2 cases of adult ophthalmia. Though unable through illness to complete his investigation, he placed the ætiological rôle of the gonococcus on a footing which has never since been seriously challenged. It was left, however, to Bumm (1885*a*, *b*) to cultivate the organism, and by inoculation experiments on human subjects to demonstrate its pathogenicity in pure cultures.

Gonorrhœa in Adults.

Gonorrhœa is an acute infectious disease, generally characterized by primary invasion of the genito-urinary tract, and frequently complicated by secondary disturbances of greater or less severity.

By far the commonest method of infection is by sexual intercourse. In man the anterior urethra is first attacked. After an incubation period of 2 to 3 days, a mucoid discharge appears, which rapidly becomes purulent. At first the gonococci are mostly extra-cellular, but soon they are taken up by the pus cells, which ingest them in large numbers; as many as 100 may be counted in a single cell.

In cases not successfully treated at this stage, the gonococci pass backwards to the posterior urethra, where they infect the glands; from this situation they are extremely difficult to dislodge. The prostate, too, is frequently infected; indeed it is generally a prostatitis which is responsible for the chronicity of the disease.

In women the disease starts with an acute urethritis; later the cervix uteri becomes infected, and may remain so for a number of years.

Complications of Gonorrhœa in Adults.—Though infection may remain localized to a small area of the genito-urinary tract, it is quite common for it to spread either by direct continuity or by the blood stream to other parts of the body, and set up complications. This may occur during either the acute or the chronic stage of the disease. Thus the gonococcus has been found in epididymitis (Colombini

1898, Witte 1900), in proctitis (Bumm 1884), in salpingitis, in arthritis (Bordoni-Uffreduzzi 1894, Neisser 1894), in tenosynovitis (Hocheisen 1906), in vesiculitis (Wynn 1905), in cystitis, in ophthalmia (Neisser 1879), less frequently in peri-renal abscesses (Colombini 1898), in pyelonephritis, and in peritonitis.

In rare instances the disease may be very acute, the organisms gaining access to the blood stream and giving rise to a bacteræmia, which may be followed by pyæmia. In these cases, which may prove fatal, there is wide-spread infection throughout the body, and post mortem there may be found endocarditis, myocarditis, less frequently pericarditis, subcutaneous and intra-muscular abscesses in different situations, polyarthritis, phlebitis, pleurisy, general adenitis, pneumonia, and septic infarcts in the spleen and kidneys. Several such cases have been reported (Leyden 1893, Ghon and Schlagenhaufer 1898, Thayer and Lazear 1899, Strong 1904, Duval and Lewis 1905, Wynn 1905, Hocheisen 1906, Jenkins 1922). Gonococci have been demonstrated in nearly all these lesions, and have often been obtained in pure culture from the blood stream both during life and after death (Thayer and Lazear 1899, Duval and Lewis 1905, Wynn 1905, Jenkins 1922). Meningitis is a rare complication (see Strumia and Kohlhas 1933). A few cases are on record of a gonococcal stomatitis (Crosby 1905), or parotitis (Colombini 1898); from the latter situation the organisms have actually been cultivated.

Gonorrhœa in Children.—Children suffer from ophthalmia neonatorum and from vulvo-vaginitis. The former is due to infection at birth with gonococci present in the maternal passages, the latter to infection of towels in infants' hospitals: but not all cases of ophthalmia neonatorum and vulvo-vaginitis are of gonorrhœal origin, and not all Gram-negative cocci found in these diseases are gonococci.

At one time ophthalmia neonatorum was responsible for a large proportion of cases of blindness, but since the introduction of the Credé preventive treatment it has become much less common.

Vulvo-vaginitis is mainly an institutional disease. Like puerperal fever and the old hospital gangrene, it is very much more frequent in institutions where large numbers of patients are collected together. Thus Fischer (1895), reporting in 1895 on the incidence during the past 2 years of the disease in the children's hospital at Altona, said that of 50 cases all but 10 were contracted in the hospital; of these 10, some had apparently been imported from other hospitals. Holt (1905) reported on no fewer than 273 cases of the disease in the Babies' Hospital, New York, during the preceding 11 years. The disease is spread from one cot to another by the use of towels and other linen that are imperfectly sterilized. Once introduced into a hospital or institution, it is a most difficult disease to eradicate; only scrupulous care in technique, constantly maintained for months on end, will prove successful eventually. The disease has a way of cropping up after it has lain dormant for weeks; to relax precautions too soon is but to court disaster. By no means all cases of vulvo-vaginitis in children are of gonococcal origin. Other organisms, such as hæmolytic streptococci and staphylococci, are often responsible, and possibly Pettit's bacillus (Hardy 1941, Osmond 1944). A diagnosis of gonorrhœal infection should therefore never be made unless the gonococcus is demonstrated. For a review of the disease, see Benson and Steer (1937).

It will be seen that the parts of the body attacked are not the same in adults as in children. Gonorrhœal ophthalmia is common in infants, rare in adults; gonorrhœal vaginitis is common in infants and children up to 5 or 6 years old, but is uncommon in adults. A similar difference is noted in the complications; thus

in adults the internal genito-urinary organs are frequently attacked ; in children these are rarely attacked, but on the other hand acute peritonitis is fairly common in female children, but rare in adults. Thus we may say that the vagina and conjunctiva are specially susceptible during infancy, and the internal genital organs during adult life. The mucosa of the urethra and rectum are susceptible throughout life. Both in adult gonorrhœa in the female, and in vulvo-vaginitis of children, the rectum is stated to be frequently involved—in 20–30 per cent. of the cases.

Reproduction of the Disease in Man.—Direct experiments on man to ascertain the pathogenicity of the gonococcus have been almost entirely confined to urethal injection. By this route, using pure cultures of the organism, it is comparatively easy to set up a typical attack of gonorrhœa, both in males and in females (Bumm 1885*a*, Wertheim 1891, Bordoni-Uffreduzzi 1894, Finger *et al.* 1895, Colombini 1898). The injection of a young culture into the urethra is followed in two or three days by a purulent urethritis, which pursues a course similar to that occurring in the natural disease, often lasting for months in spite of treatment. Gonococci are present in the pus cells, and may be recovered in pure culture.

Not only is it possible to convey the disease to a normal person by injection of pure cultures, but it is possible to incite an acute attack in a patient who is suffering from chronic gonorrhœa. There is apparently little, if any, immunity acquired to the disease. Injection into the human urethra of killed gonococci gives rise to a transitory purulent inflammation (Scholtz 1900).

Steinschneider and Schäffer (1895) injected large numbers of living gonococci into the subcutaneous tissues of healthy persons, and found that no reaction of any sort occurred.

Using gonotoxin, de Christmas (1897) found that the injection of 1 ml. into the human urethra gave rise to a urethritis, coming on in 2 hours, and subsiding after about 2 days. No immunity was established ; the same effect was reproduced after each of 5 successive injections. Wassermann (1898) injected 0·1 ml. of toxin subcutaneously into himself ; 4 hours later a reaction set in, characterized by local inflammation, slight shivering, an evening rise of temperature, headache, and pains in the limbs and joints. He then injected the same dose subcutaneously into two patients suffering from chronic gonorrhœa ; similar results followed, accompanied by swelling of the local glands. No immunity to the toxin was established ; the same reaction was repeated after 5 separate injections at 4-day intervals.



FIG. 282.—*Neisseria gonorrhœæ* in film of pus from acute gonorrhœa (× 1000).

Diagnosis.

The diagnosis of gonorrhœa depends in the main on the demonstration of the causative organism. The characteristic morphology and staining reaction of the gonococcus, and the fact that a considerable proportion of the cocci are always intracellular, make it possible to confirm the clinical diagnosis by microscopical examination of the purulent discharge in *acute* infections (Fig. 282). Wherever any element of doubt exists, cultures should be prepared on

a suitable medium (see Chapter 23). In *chronic* cases it may be extremely difficult to demonstrate the gonococcus in films, or to isolate it in culture. This holds particularly true for the female, where a diagnosis on microscopic grounds alone can rarely be made. Appropriate measures must be taken to obtain material for examination from the situation most likely to be the seat of chronic infection—the deep urethral glands and the prostate in the male, the Bartholinian glands and the cervical glands in the female. It may be noted that the fluid withdrawn from the joints in gonorrhœal arthritis is frequently sterile, and that the same is true of pus withdrawn from the Fallopian tube in chronic cases of gonorrhœal salpingitis.

For diagnostic cultivation the method described by McLeod and his colleagues (1934) is to be strongly recommended. The medium used is a 10 per cent. heated blood agar prepared from broth made according to Wright's (1929, 1933) method, *i.e.*, extraction of the minced meat for 45 minutes at 60° C. in the presence of 1 per cent. peptone and 0·2 per cent. Na_2HPO_4, followed by steaming. The broth should have a reaction of pH 7·4, and the minimum amount of agar necessary to give stability to the medium should be added. The plates are first incubated for 18 hours at 36° C. in a closed jar containing air, 8 per cent. of which has been replaced by CO_2, and then for 24 hours under ordinary aerobic conditions. A 1 per cent. solution of tetramethyl-*p*-phenylenediamine hydrochloride is then poured over the plate and run off immediately. The effect of this solution is to turn gonococcal colonies a bright purple colour. Medium-sized convex translucent colonies that rapidly turn bright purple and are found microscopically to consist of Gram-negative diplococci may be provisionally accepted as gonococci. For further identification, when necessary, the colonies are picked off, and studied culturally and biochemically. A rapid provisional method for establishing their identity based on the solubility of gonococci in N/10 NaOH is described by Cantor, Shelanski and Willard (1942). Occasionally colonies giving the oxidase reaction and consisting of Gram-negative diplococci are isolated from the genito-urinary tract which subsequent examination shows are not true gonococci. Organisms of this type are unlikely to give rise to more than 1·5 per cent. of erroneous diagnoses. Cultural methods are greatly superior to direct microscopical examination of smears (see, for example, Gibbons 1937, Carpenter, Leahy and Wilson 1938, Sewell, Clarke and Nelson 1941); nevertheless a certain number of cases do occur in which the smear method is positive when the cultural method is negative. The greatest caution, however, must be taken in chronic cases in identifying the gonococcus on morphological appearances alone. As Beck (1933) points out, confusion is often caused by staphylococci and streptococci, which may occur in pairs and fail to retain the Gram stain. If swabs for cultural purposes cannot be inoculated at once on to a suitable medium, measures must be taken to prevent death of the gonococci. Cox and McDermott (1943) recommend collection of the exudate on to a small wooden toothpick; this is dropped into a tube, 50 × 6 mm., containing 0·6 ml. of a mixture made up of equal parts of sterile distilled water and defibrinated horse blood to which 1/30,000 crystal violet and 5 mgm. per 100 ml. of *para*-aminobenzoic acid are added. The tube is closed with a corked paraffined stopper. By this means successful results may be obtained, even after transmission through the post.

In chronic or latent gonorrhœa, or in cases of rheumatism, iritis, etc., in which a latent gonococcal infection is suspected, failure to demonstrate the presence of the gonococcus can never be regarded as definite evidence that this organism is absent from the tissues. In such cases certain indirect methods of diagnosis are available.

The Complement-Fixation Test.—Müller and Oppenheim (1906) were the first to apply this test to clinical practice, obtaining a positive result in a case of gonorrhœal arthritis. Since then it has been used by a large number of workers (Watabiki 1910,

Schwartz and McNeill 1911, 1912, Keyes 1912, Kolmer and Brown 1914, Irons and Nicoll 1915, Dixon and Priestley 1919, Priestley 1919, Magner 1920, Smith and Wilson 1920, Herrold 1921, Torrey and Buckell 1922, Torrey *et al.* 1922, Walker 1922, Rubenstein and Gauran 1923, Osmond and Oliver 1929, Kristjansen 1931, Price 1933, Thjøtta and Waaler 1933, Schröpl 1934).

The technique of the reaction requires extreme care, and for a detailed description of the various methods employed the student is referred to the Medical Research Council's Report (1918)—a report which has been largely drawn on in the present section on diagnosis. It is generally recognized that a polyvalent antigen and an anti-sheep hæmolytic system are most satisfactory.

Great care is necessary in the interpretation of the reaction. The following points must be borne in mind: (1) the reaction does not become positive for at least 2 weeks after infection; (2) it is often negative in simple cases of anterior urethritis in the male, and of urethritis in the female; (3) it is generally positive in cases in which complications are present; (4) it remains positive for about 3 months after clinical cure; (5) a single negative reaction is of little importance; (6) two or three successive negative reactions during the course of 4 to 6 weeks are of considerable importance in excluding the presence of the disease; (7) a positive reaction becoming negative about 3 months after clinical cure is strong evidence in favour of real cure; (8) a positive reaction denotes the recent activity in the body of a focus of living gonococci.

Sera of patients who have never had gonorrhœa are said to be consistently negative. The injection of vaccines interferes with the test, as the gonococcal antigen itself is able to stimulate the production of specific immune bodies; the reaction is therefore valueless in vaccine-treated patients. Only in one other disease may the reaction be positive—namely in cerebrospinal meningitis; the two diseases are so easily differentiated on clinical grounds that confusion is unlikely.

Table 125 shows the percentage of positive reactions which may be expected in certain gonococcal infections. It has been compiled largely from the results of Schwartz and McNeill (1911, 1912), Kolmer and Brown (1914), Smith and Wilson (1920), Walker (1922), Torrey, Wilson and Buckell (1922), and Rubenstein and Gauran (1923).

It is seen that complications in the male genital system and in the joints give the highest percentage of positive results. The test is therefore of considerable value in those cases in which microscopic and cultural tests are most likely to fail. It is of especial value in chronic pelvic disease.

The complement-fixation test is being regarded with increasing favour. The more recent reports record a very small proportion of false positive reactions—usually under 1 per cent.—and a higher proportion of positive results than is given in Table 125. Price (1933) in particular, using a modified technique, claims to have obtained a very high proportion of positive results, ranging from 27 per cent. in the 1st week after exposure to 100 per cent. in the 5th week.

The figures given by Tulloch (1929) from Dundee may also be quoted.

Complement fixation	positive:	microscopical findings	positive:	433
„	„	negative:	„	„	negative: 737
„	„	positive:	„	„	negative: 163
„	„	negative:	„	„	positive: 215

The figures given in the last row refer mainly to early cases in which the complement-fixation reaction had not had time to become positive. Those in the

TABLE 125

Acute Gonorrhœa, 2–4 weeks	48%	positive	reactions
Subacute and Chronic Gonorrhœa	61%	„	„
Epididymitis	82%	„	„
Prostatitis	80%	„	„
Vesiculitis	88%	„	„
Metritis	68%	„	„
Salpingitis	77%	„	„
Vulvo-vaginitis	50%	„	„
Arthritis	82%	„	„

third row refer mainly to chronic cases, and it is in such cases that the complement-fixation test finds its main value.

Precipitin Reaction.—This method was advocated by Robinson and Meader (1920) who found that discharges from gonorrhœal inflammations gave a precipitin reaction with antigonococcus serum, even when no gonococci were to be found microscopically. Kelley (1922), who tried this reaction, was unable to confirm their results. The test cannot yet be relied on for diagnosis.

More recently Meinicke (1931) has introduced his *Klarungsreaktion* (MKR test) and Müller (1932) his *Ballungs-Reaktion* (Im.B.R. test) for the diagnosis of gonorrhœa. According to Schröpl (1934), who made comparative observations on these tests, both of them give a higher proportion of non-specific reactions than the complement-fixation test, especially with syphilitic sera. One or other of them may be used, however, in conjunction with the complement-fixation reaction in order to amplify and control the results obtained by this test.

Skin Reaction.—Bruck (1909) found that patients with gonorrhœa showed a marked skin reaction to dead gonococci. Kohler employed this test in diagnosis, and found it to be valuable in many cases.

Irons (1912) prepared a glycerinated suspension from several strains of gonococci, which he called gonococcin. Inoculation was made on the skin with the point of a needle. After a few hours, in positive cases, a papule appears, surrounded by hyperæmia, reaching its maximum size in 24 hours, and disappearing after several days. The diameter of the papule and the hyperæmic zone around it is 5 mm. or more. The reaction was found to be generally positive at some time during the course of the disease, but in cases of severe infection, it might remain consistently negative. On the whole, it does not appear to be sufficiently regular to be of value in diagnostic work.

Prevention and Treatment.

Prevention is intimately associated with early diagnosis and efficient treatment. The mode of infection indicates sufficiently clearly the ways in which the disease can be avoided, and the reservoirs of infection which exist in any community. The problem is one of personal and social hygiene.

Opinions as to the value of vaccine treatment differ considerably. The evidence available does not lend itself to statistical analysis (see Aronstam 1908, Ballenger 1908, Butler and Long 1908, Churchill and Soper 1908, Hamilton and Cooke 1908, Bruck 1912, Lees 1919, Thomson 1919).

Antibacterial sera have been employed in the treatment of gonorrhœa, but without any conspicuous success. The infection is not of a type in which we should expect such sera to be of value (see Rogers 1906, Torrey 1906, Jötten 1921).

Of recent years the whole problem of treatment has been altered by the introduction of the sulphonamide group of drugs and penicillin. Of the sulphonamides, sulphathiazole and sulphadiazine have so far given the best results. In males,

4 days' treatment with 5 gm. of sulphathiazole a day is said to cure over 90 per cent. of acute cases (Harrison 1942; see also Osmond 1943). Early acute cases in females likewise respond well to treatment, but chronic cases present far greater difficulty (Osmond 1944). In spite, however, of the outstanding success of the sulphonamides, numerous failures are experienced. The cause of these is not perfectly understood. In some instances the infecting strain is resistant to sulphonamide or becomes resistant as the result of insufficient dosage; in others the natural defence mechanism appears to be inadequate; and in others there is a suggestion that the patient's serum possesses properties antagonistic to sulphonamide action (see Editorial 1944). Fortunately most cases that prove resistant to sulphonamide treatment respond well to penicillin. Acute cases may be cured by the intramuscular injection of about 20,000 units every 3 hours till five doses have been given. Gonococci disappear within a few hours of the beginning of treatment, and within 24 hours the urethral discharge dries up completely. How far penicillin treatment will prove successful in chronic cases, and whether penicillin-resistant strains will increase as this form of treatment becomes more prevalent, it is as yet impossible to say. It may be noted that treatment with penicillin is now the method of choice in gonorrhœal ophthalmia

One further method is available, namely that of induced pyrotherapy. This depends on the fact that in culture the gonococcus is almost completely destroyed by exposure for 2 hours to a temperature of 41·5°–42° C. (see Carpenter *et al.* 1933). The patient's temperature is raised by placing him in a special cabinet heated with carbon filament lamps, or by passing high-frequency currents through his trunk. The aim is to keep his temperature at a sufficiently high level for a sufficient length of time to bring about *in vivo* sterilization of the gonococci. This is a very severe form of treatment, not without its hazards (Wallace and Bushby 1944), but it holds out hope for the patient when other methods have failed (Warren *et al.* 1937).

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CHAPTER 66

SCARLET FEVER, AND OTHER DISEASES DUE TO HÆMOLYTIC STREPTOCOCCI

SCARLET FEVER

Our present conception of the mechanism of infection and resistance in scarlet fever affords an excellent example of the solution of a bacteriological problem by the convergence of evidence obtained along many different lines of investigation.

The fact that long-chained streptococci are frequently present in the throat during the early stages of scarlatinal infection has been known since the early days of bacteriology. Baginsky and Sommerfeld, in 1900, recorded their presence in each of 700 cases. As soon as the differentiation of streptococci into hæmolytic and non-hæmolytic types had been established, it became clear that the species so constantly associated with scarlet fever was *Str. pyogenes*. The streptococcal infection appears not to be strictly confined to the tonsils or fauces; Hektoen, in 1903, reported the isolation of streptococci from the blood in 12 per cent. of the cases which he studied, and noted that this evidence of bacteræmia was not necessarily of bad prognostic significance. In spite of such observations as these, there was great hesitation in accepting the undoubted association between hæmolytic streptococci and scarlet fever as one of cause and effect; and this hesitation was natural. *Str. pyogenes* was already known as the essential cause of various acute suppurative and septicæmic infections in man; and there seemed no means of differentiating between the strains isolated from scarlet fever, and those isolated from such conditions as cellulitis or erysipelas. A septic form of scarlet fever was well recognized by clinicians, in which hæmolytic streptococci were by general consent accorded the principal causative rôle, and it was commonly agreed that these organisms were the most frequent causative agents in such complications as otitis media; but the general trend of opinion was to regard *Str. pyogenes* as an important secondary invader, playing a part similar to that of *Str. pneumoniæ*, or of *Str. pyogenes* itself, in the secondary pneumonia of measles, influenza, or whooping cough. Moreover, the bacteriological findings had, in some directions, an appearance of inconsistency. Jochmann (1905), for instance, reported his failure to isolate streptococci from the blood, or tissues, of patients dying within a few days from a malignant form of the disease.

The suspicion remained, that the association might be closer than that implied by the dominant view; and, whenever a new method of bacterial differentiation was applied to the *Streptococcus* group, an attempt was made to compare scarlatinal strains with those derived from other sources. The use of fermentation tests failed to distinguish any group which was constantly associated with scarlet fever; though some observers recorded a high frequency of particular fermentative types

in association with this disease. The introduction of serological methods of differentiation seemed to offer a better hope of solving this problem, and Moser and von Pirquet (1902), Meyer (1902) and Rossiwall and Schick (1905) reported that scarlatinal strains of hæmolytic streptococci could be differentiated by agglutination tests. These findings were, however, not confirmed by other observers (Neufeld 1903, Aronson 1903, Hasenknopf and Salge 1903); and the problem remained unsolved. In the light of our present knowledge we can hazard the guess that, along these lines alone, it would have remained insoluble. It is true that the renewed interest in the antigenic analysis of the hæmolytic streptococci which followed on the studies of Dochez, Avery and Lancefield (1919) led to the collection of important new data, and that the results recorded by Bliss (1920, 1922), Gordon (1921), Eagles (1924), and Stevens and Dochez (1926*a* and *b*) went far to confirm and extend the earlier observations of Moser and von Pirquet; but the more detailed studies, which were undertaken in the years before the war by Griffith (1926, 1927, 1928, 1934, 1935), Smith (1926, 1927*a*), James (1926), Gunn and Griffith (1928), McLachlan and Mackie (1928) and others (see Chapter 24), revealed a high degree of antigenic heterogeneity among the hæmolytic streptococci, and made it abundantly clear that there was no one antigenic type to which the name *Str. scarlatinæ* or *Str. pyogenes* var. *scarlatinæ* could be applied, though some types were far more commonly associated with scarlatinal infections than others, just as some types of pneumococci are isolated more frequently than others from cases of lobar pneumonia. Practically all strains of hæmolytic streptococci that have been isolated from cases of scarlet fever have been found to belong to Lancefield's Group A (*Streptococcus pyogenes*), the strains falling within this group being characteristically pathogenic for man (see Lancefield 1928, and, for further details of antigenic structure, Chapter 24). Of a total of 8,096 strains reviewed by Schwentker, Janney and Gordon (1943*b*), 7,995 belonged to Group A, 2 to Group B, 39 to Group C, 21 to Group G, and 39 to unidentified groups. Thus, about 99 per cent. of all strains fell into Group A. The isolation of occasional strains of Group C or G need occasion no surprise, since these organisms are not infrequent in the normal throat.

In Britain, Types 1, 2, 3 and 4 were most frequently isolated from cases of scarlet fever by Griffith (1934) (see also Cooke and Neisser 1937, Green 1937, Neisser 1939, de Waal 1940, 1941). In Gelsenkirchen, Germany, Gundel and Wustenberg (1937) found Types 4, 3, 5, 1 and 2. In Tokyo, Kodama and his colleagues (1937, 1939) record predominance of Types 1–4 in the winter of 1936–37, of Types 2, 4, 8 and 10 in 1937–38, and of Types 2, 4 and 8 in 1938–39. The common types in Australia are different. In Sydney, White, Rudd and Ward (1939) found that Types 17, 1, 4, 6, 5, and a local type "Wade" predominated in 1936, and Types 11, 4, 17, "Wade" and 1 in 1938. Keogh and his colleagues (1939*a*) could find no Types 1, 2 or 3 in Adelaide, where Type "Wade" predominated; in Melbourne, however, Type 2 predominated in 1939–40, the remainder of the cases being associated with Types 17, "Wade," 11 and 6 (Kelsey 1941, Keogh, Simmons and Wilson 1941). The American figures are again different. Bailey (1939), for example, in Chicago found Types 2, 3 and 6. In a general survey of scarlet fever streptococci in the U.S.A., Keefer and his colleagues (1942) found that Types 15, 13, 11, 1 and 2 predominated in 1938–39; and among soldiers in a U.S. Army camp, the scarlet fever types were 3, 17, 19 and 1 (Hamburger *et al.* 1944). Bynoe (1943) records that in Ottawa, 1940–42, the prevalent types were 3, 19 (17), 4, 2, 6 and 14.

Most observers are agreed that the scarlet fever types in a given community also predominate in other varieties of streptococcal infection—tonsillitis, pharyngitis, puerperal

fever and so forth—but occasionally there are marked differences. Thus Hamburger and his colleagues (1944) noted that Type 6 was, among others, prevalent in throat infections in a camp, but never gave rise to scarlet fever. As regards the severity of the scarlet fever produced by different types, no generalization is possible, though in some communities, certain types—for example, Type 1 in Britain, and Type " Wade " in Australia —have been found to be associated with the severer infections.

Once it had been shown that *Str. pyogenes* was constantly associated with scarlet fever it was natural to inquire whether this organism would reproduce the disease in any experimental animal. The results obtained when the usual laboratory animals were inoculated with cultures of hæmolytic streptococci, from any source, showed that the guinea-pig, rabbit and mouse differed in their resistance to this bacterial species, and that the type of infection produced varied in relation to the virulence of the particular strain inoculated, and the route of inoculation; but there was no indication of any correlation between a particular type of experimental infection and the source from which the infecting strain was obtained; nor did any of the inoculated animals develop lesions which bore any obvious relation to scarlet fever in man.

Dochez and Sherman (1922), however, found that the subcutaneous inoculation of guinea-pigs with streptococci embedded in agar gave rise to a fever accompanied by an erythematous rash, which later desquamated. Landsteiner, Levaditi and Prasek (1911) produced scarlet fever in monkeys by inoculation of the throat with faucial exudate from scarlatinal patients. *Str. pyogenes* was recovered from the fauces of animals so infected, but these strains failed to reproduce the disease in other monkeys.

The proof that scarlet fever is caused by infection with hæmolytic streptococci rests on a series of observations that have been made on man himself, and these observations have elucidated the pathogenesis of the disease, as well as its causation.

In 1914 Krumwiede, Nicoll and Pratt recorded an instance in which a laboratory worker was accidentally infected by sucking into her mouth a suspension of living streptococci. After an incubation period of three days she passed through a typical attack of scarlet fever. In 1921 Dick and Dick attempted to reproduce scarlet fever in a series of human volunteers by inoculating the throat with various organisms isolated from scarlatinal patients, including hæmolytic streptococci; none of these volunteers developed the typical disease, though some developed tonsillitis. In 1923 the same observers carried out another series of experiments, using a strain of *Str. pyogenes* which had been isolated from the finger of a nurse who was suffering from surgical scarlatina. Of five volunteers, who were inoculated by swabbing the fauces with a broth culture of this organism, three showed no reaction, one developed a sore throat with fever, but without rash, and one passed through a typical attack of scarlet fever. Five other volunteers, who were swabbed with filtrates from these cultures, showed no reaction. When these were later swabbed with living cultures, one of them developed scarlet fever. Toyoda and others (1931) record the production of scarlet fever in three volunteers by swabbing the throat with hæmolytic streptococci from scarlatinal patients.

It has thus been shown that certain strains of *Str. pyogenes* are capable of causing typical scarlet fever in susceptible human beings; but these susceptible individuals are in a minority among any random sample of the adult population.

The explanation of this unequal distribution of resistance has emerged from a series of studies carried out along somewhat different lines. In 1918 Schultz

and Charlton recorded a phenomenon which has played an important part in the subsequent study of the disease. They found that 1 ml. of normal human serum, injected into the skin of a patient displaying a characteristic scarlatinal rash, produced a blanching of the rash in the immediate neighbourhood of the injection. This property was shared by the serum from a patient who was convalescent from scarlet fever, but not by serum withdrawn from a patient during the height of an attack. It has since been shown that the Schultz-Charlton phenomenon is given by about 60 per cent. of normal sera (Dochez 1925). This reaction was originally regarded as the result of the presence, in normal serum, of some substance which was removed from it during an attack of scarlet fever; but Mair (1923) recorded an instance in which he was able to demonstrate that the serum of a child gave a negative Schultz-Charlton reaction before an attack of scarlet fever, and a positive reaction after convalescence. He noted that sera obtained from young children, who have not had scarlet fever, give a much higher proportion of negative reactions than sera from a random sample of adults; and he put forward the view that the positive reaction is the result of the development of a specific antitoxin, which neutralizes the toxin present in the skin of a scarlet fever patient. According to this view the rash, and probably other features of the scarlatinal syndrome, are to be regarded as toxæmic in origin.

It had long been known that certain strains of hæmolytic streptococci yielded filtrates which, in large doses, were toxic for laboratory animals: but the susceptibility of these animals was so slight that little or no progress was made in the study of such toxic filtrates before the investigation by Dick and Dick (1924*a*, *b*, 1925*a*, *b*) of their action in man. Their results showed that Berkefeld filtrates of broth cultures of scarlatinal strains of *Str. pyogenes* contained a toxic substance which produced a typical erythematous reaction, when injected intradermally, in a proportion of normal persons who had not suffered from scarlet fever (see p. 591). This local reaction was induced by the injection of 0·1 ml. of a 1 : 1,000 dilution of the particular toxic filtrate employed. This test, which is more fully described on p. 1467, has since been applied to large samples of the normal population; and the distribution of positive and negative reactors, which is closely analogous to that revealed by the application of the Schick test in the case of diphtheria, is in full accord with the view that the great majority of young children are susceptible to the erythrogenic toxin, but, with advancing years, acquire an active antitoxic immunity, either by passing through an attack of the clinical disease, or, more commonly, by an immunizing infection with a toxin-producing streptococcus without the production of the characteristic scarlatinal syndrome (see Chapter 49 and Zingher 1924).

The administration of larger doses of erythrogenic toxin to susceptible persons has yielded even more demonstrative results. In a number of cases such doses have induced a generalized reaction with fever, nausea, vomiting and a transient scarlatiniform rash. Most of the recorded instances of this "miniature scarlet fever" were observed during the earlier stages of the study of the erythrogenic toxin; but it still occurs occasionally, when a particularly susceptible person is undergoing immunization with doses of the ordinary size (see p. 1473). This reaction recalls the observation of Gabritschewsky (1907), who, during the inoculation of a large series of persons with a killed culture of scarlatinal streptococci, noted a scarlatiniform reaction in a number of his subjects. It did not occur in persons who had had scarlet fever, nor in persons injected with anti-scarlatinal

serum prepared (Moser 1902) by inoculation of horses with scarlatinal streptococci (see also Szirmai 1942).

Additional evidence has been contributed by the studies of Trask and Blake (1924), who record the following significant observations. A toxic substance can be demonstrated in the serum of patients acutely ill with scarlet fever, which produces a typical skin reaction when injected intracutaneously into persons who have not had scarlet fever, and whose serum gives a negative Schultz-Charlton reaction (*i.e.* contains no antitoxin). Such injections produce no reaction in persons whose serum gives a positive Schultz-Charlton reaction. This toxic substance is not neutralized by a human serum which gives a negative blanching test, but is readily neutralized by a human serum which gives a positive blanching test. It is not neutralized by normal horse serum, but it is readily neutralized by a horse anti-scarlatinal serum.

We may also note here that during the course of scarlet fever there is usually evidence of a serum response to other products of *Str. pyogenes*. Recovery is associated with an increase in the resistance of the plasma to streptococcal fibrinolysin (Tillett and Garner 1933, Tillett, Edwards and Garner 1934, Stuart-Harris 1935, Bau and Kleu 1936, Waaler 1936, Gordon and Balteanu 1937) and with an increase in antibody to the O streptolysin of *Str. pyogenes* (Todd 1932, Longcope 1936, Green 1941, Mote and Jones 1941, Rantz, Kirby and Jacobs 1943). Rantz and his colleagues also noted that in 13 of 24 patients with scarlet fever there was a significant rise in agglutinins for the infecting type of streptococcus.

Taking this evidence as a whole, there seems little doubt as to its import. Scarlet fever in man is a disease caused by infection with *Str. pyogenes*. The causative organisms are, for the most part, localized in the throat, though there may be a transient bacteræmia in some cases. The clinical manifestations of the disease result from the action of a soluble toxin, which is absorbed from the local lesion and carried to the susceptible cells and tissues. The varying susceptibility of children and adults, and of the same age groups in different social environments, depends upon the presence or absence of circulating antitoxin. Animals other than man, and perhaps certain anthropoid apes, do not reproduce the disease, even when experimentally infected, because of their much greater resistance to the scarlatinal toxin.

Only one other point requires consideration at this stage, the unity or diversity of the toxins produced by different strains of *Str. pyogenes*. In much of the earlier work there was an overt or implicit suggestion that the scarlatinal toxin was a characteristic and distinctive product of those races of hæmolytic streptococci which were associated with scarlet fever. Subsequent studies have shown that this is not the case. Omitting any detailed descriptions of the results recorded by individual investigators (Park and Spiegel 1925, Lash 1926, Kirkbride and Wheeler 1926, Eagles 1926, Smith 1927*b*, McLachlan 1927, Okell and Parish 1928), the evidence at present available tends to support the following conclusions. The production of a soluble exotoxin, giving typical skin reactions in man, is characteristic of *Str. pyogenes* in general, and not of scarlatinal strains in particular. There is, however, some evidence that toxigenicity is a more constant character in scarlatinal than in non-scarlatinal strains. Qualitative differences in the toxins formed by different strains of Group A streptococci have been described by Wadsworth and Coffey (1935), who studied toxins made from 314 strains. Of these, 77·4 per cent. were neutralized by one monovalent antistreptococcal goat serum, 10·5 per cent. by a second, and 10·5 per cent. by a third serum. The remaining 1·6 per cent. of toxins were neutralized by a combination of the sera. Fraser (1937) reported similar results. It would appear that ordinary commercial antitoxin should neutralize a high proportion of all toxic strains. Whether an erythrogenic toxin is produced by any other species, or group, of hæmolytic streptococci is as yet uncertain (see Chapter 24).

The Bacteriological Diagnosis of Scarlet Fever.

Scarlet fever, in its characteristic clinical form, is a disease in which a throat infection with *Str. pyogenes* is associated with a rash produced by the erythrogenic toxin, and a syndrome that is, in the main, toxæmic in origin. A diagnosis of a throat infection caused by *Str. pyogenes* is not therefore a diagnosis of scarlet fever; indeed, a clinician will frequently be concerned in distinguishing a throat infection of this type from a case that, because of other criteria, would fall into the scarlet fever category. A throat swab has not, under these conditions, any great diagnostic value. We should expect to isolate *Str. pyogenes* from every case of scarlet fever; but we should also expect to isolate it from many cases to which that diagnosis would not be applicable. It may be noted that the attempt to isolate *Str. pyogenes* from genuine cases of scarlet fever is by no means uniformly successful. In our own experience about 10 per cent. of throat swabs taken during the first three or four days of the disease have failed to yield this organism, though we have almost invariably isolated it during the second half of the first week. As a routine it is advisable to inoculate two blood agar plates and one gentian violet blood agar plate, and to incubate one of the blood agar plates anaerobically in order to favour hæmolysis. Very occasionally, *Str. pyogenes* fails to form hæmolytic colonies, and its recognition becomes particularly difficult (Coburn and Pauli 1941, Colebrook *et al.* 1942).

The Diagnosis of Susceptibility or Immunity to Scarlet Fever.

For this purpose we use the Dick test, in which a small dose of toxin is injected into the skin of one arm, and a dose of inactivated toxin into the skin of the other, to serve as a control. The amount of toxin injected is 0·2 ml., containing one skin-test dose, and the method of performing the test is essentially the same as in the Schick reaction (see p. 1376). The following differences should, however, be noted.

The erythrogenic toxin is far more heat resistant than diphtheria toxin. The filtrate that is to serve as a control must therefore be heated at 96° C. for 45 minutes. A control test should never be omitted (see Burton and Weir 1944).

The skin reaction to the erythrogenic toxin occurs more rapidly than the reaction to diphtheria toxin. A bright red flush appears within 6 to 12 hours, attains its maximum in about 24 hours, and then fades rapidly, so that it may be almost invisible on the third day. The pseudo-reaction, when it occurs, is smaller and less intense than the true positive reaction and fades more slowly. In this last respect, the time relations of the positive and pseudo-reactions in the Dick test are the reverse of those in the Schick test, and the times of reading the Dick test must be adjusted accordingly.

The interpretation of the test does not differ from that of the Schick reaction. A positive reaction (positive on the test arm and negative on the control) or a combined reaction (positive and pseudo on the test arm and pseudo on the control) indicates susceptibility. A negative reaction (negative on both arms), or a pseudo-reaction (pseudo on both arms), indicates immunity.

We can safely assume that, except in young infants (see p. 1078), a negative reaction indicates the presence of antitoxin in the circulation or the tissues; this has been shown to be the case in many instances, and the Schultz-Charlton reaction affords further proof of the presence of antitoxin in the serum of resistant persons. Because of the difficulty of titrating scarlatinal antitoxin, to which we shall refer in a later section, we have, however, much less information in regard

to the level of antitoxin that confers an effective immunity than we have in the case of diphtheria.

Although the reactions to the Schick and Dick tests have the same general significance, experience has shown that they are not equally reliable. It is, for instance, uncommon for any Schick-negative person to develop diphtheria, while almost every case of diphtheria is found to be Schick-positive at the onset of the disease. In the case of the Dick test the figures, though tending quite clearly in the same direction, are of a different order.

Thus Toyoda and his colleagues (1929) note that 41 of 305 cases of scarlet fever (14 per cent.) were known to be Dick-negative shortly before infection, They give the proportion of cases that are Dick-negative during the early stages of the disease as 23–59 per cent., according to different observers, and the proportion negative during convalescence as 82·3–91·7 per cent. Okell (1932) records similar observations—22 Dick-positive reactors among 119 cases of scarlet fever 3 to 12 weeks after the onset of the disease—and notes that a considerable proportion of persons who have had scarlet fever in early life and are almost certainly immune give positive reactions. Freudenberg (1941) Dick-tested 303 children 6 weeks after the onset of an attack of scarlet fever, and found that only 38 per cent. of 236 children aged 0–10 years were Dick-negative, whereas 63 per cent. of 67 children aged 10–16 years were negative. The association of immunity with a negative Dick-reaction was clearly shown in the incidence of a second attack in 1200 children, of which the Dick-tested children were a sample. Of the 19 second attacks observed, 18 occurred in the 0–10 year age group, and one only in the older children.

It would seem a just conclusion, from the evidence at present available, that a Dick-negative reactor is much less likely to contract scarlet fever than a Dick-positive reactor, but that the odds against his doing so are not nearly so high as the odds against a Schick-negative reactor developing diphtheria, and that similarly a Schick-positive reaction is a more reliable indication of susceptibility to diphtheria than is a Dick-positive reaction to scarlet fever. These conclusions should perhaps be amplified by pointing out that, so far as susceptibility to streptococcal throat infection is concerned, there seems to be no difference between Dick-positive and Dick-negative reactors (Schwentker *et al.* 1943*a*, *b*, Kidd 1944). Both types of reactor appear to be equally liable to become symptomless carriers; and the incidence of complications appears to be the same in patients with and without a rash (Hobson 1936, Stebbins *et al.* 1937). The only difference is that a positive reactor is more likely to develop a rash than a negative reactor. Even so, it is a common experience to observe the absence of a rash in Dick-positive persons infected with a strain known to produce erythrogenic toxin.

The Standardization of Erythrogenic Toxin.—One of the great difficulties of the Dick reaction is that involved in the standardization of the erythrogenic toxin (see Okell 1932, Friedman *et al.* 1935). The skin test dose is defined as the smallest amount of toxin that will, on the average, produce an erythematous reaction, 1 cm. in diameter, in the skin of a susceptible person within 24 hours. This dose, in fact, often corresponds approximately to 0·2 ml. of a 1 : 1,000 dilution of a toxic broth culture. Its accurate measurement is, however, almost impossible; in part, because the assumption that any sample of human beings are fully, or equally, susceptible, even when all react positively to a particular dilution of toxin, is quite unjustified; in part, because an adequate supply of susceptible persons on whom the tests can be performed is not easy to obtain. When several fully susceptible persons are available for the titrations, an erythrogenic toxin can

be measured with an approximation to accuracy—a filtrate of strength x can, for instance, be differentiated from a filtrate of strength $2x$— but it is not always easy to do even as well as this (see, for instance, James, Joe and Swyer 1929, O'Brien 1930, Kolchin 1940). This question will be discussed in more detail when we are considering the standardization of antitoxin.

The Epidemiology of Scarlet Fever.

There is every reason to suppose that the epidemiology of scarlet fever is closely analogous to that of diphtheria. In both cases we have diseases characterized by primary throat lesions and a characteristic clinical syndrome that is determined, in the main, by the effects produced by the diffusion of a soluble toxin from the primary lesion. In both, carriers of the causative organism greatly outnumber the clinical cases. In both, the population at large is undergoing, from early childhood onwards, an active immunization resulting from latent or atypical infections (see, for instance, Okell and Parish 1928, Stocks 1930, Allison and Gunn 1932, Okell 1932). They differ, however, in one important respect. Broadly speaking, diphtheria is the only clinical manifestation of *C. diphtheriæ*, whereas scarlet fever is but one manifestation of a wide variety of lesions caused by *Str. pyogenes*.

The earlier observations on the distribution of the causative organism, including those carried out in the authors' laboratory at Manchester and in London (Report 1930, Straker *et al.* 1939), referred to the incidence of hæmolytic streptococci in general, but more recent information is available on the incidence of *Str. pyogenes* in particular. The frequency with which this organism can be isolated from the throat of normal persons not in contact with epidemic streptococcal infection of the respiratory tract varies widely with age, season, country, occupation and doubtless other factors. Thus the following figures may be quoted as examples: healthy children and adults in Melbourne 4·5 per cent. (Keogh *et al.* 1939*b*); school children in Melbourne 13·3 per cent. (Macdonald *et al.* 1940); students, staff and out-patients in hospital at Patna 10·6 per cent. (Chatterjee and Mitter 1941); parturient women in Shanghai 1·8 per cent., and hospital nurses in Shanghai 4 per cent. (Wu 1941); healthy girls in a residential school in Buckinghamshire 4·2 per cent. (Report 1941); patients suffering from measles on admission to hospital at Bristol 11·7 per cent. (Peters *et al.* 1942); Army recruits in Wyoming 8 per cent. (Schwentker 1943); incoming naval recruits in the United States 4 per cent. (Schwentker *et al.* 1943*a*); Bantu children 19·0 per cent. (Murray 1943); patients and nurses in the Birmingham Accident Hospital 19·2 per cent. (Williams and Harper 1944).

On the whole, the carrier rate in the throat of normal persons usually seems to vary between 4 and 8 per cent. It tends to be highest in the winter and spring, and lowest in the summer and autumn months.

In any community, such as a school, where overt infections like tonsillitis or scarlet fever are occurring, even though the number of such cases is small, the incidence of *Str. pyogenes* in the throat tends to be far higher. Carrier rates of 25 or 50 per cent., or even more, are by no means uncommon. In the nose *Str. pyogenes* is much less frequent than in the throat. The normal incidence is below 1 per cent. When it is present in the nose, it is almost invariably found in the throat as well.

We have described on p. 1387 the way in which diphtheria bacilli disappear from the throat during convalescence, and reference to Table 115 will show that about 50 per cent. of diphtheria convalescents have ceased to carry by the 20th day, and 90 per cent. by the 50th day. It has become quite clear that the disappearance of hæmolytic streptococci from the throat during convalescence from scarlet fever is

much slower (see Nicholls 1927, Gordon 1927, Gunn and Griffith 1928, Allison and Gunn 1932, Brown and Allison 1935, Allison and Brown 1937, de Waal 1940); and it would seem that the carrier rate among convalescents on discharge from hospital is usually of the order of 50 per cent. By itself, however, this statement is misleading, unless it is pointed out that the carrier strain on discharge is often different from that on admission. The observations of Allison and Brown (1937), followed by those of numerous other workers (Cooke and Neisser 1937, de Waal 1940, Peters *et al.* 1942), have shown that a variable proportion of scarlet fever patients treated in general wards become cross-infected with a strain of a different serological type. This happened, for example, in 33 out of 47 of Allison and Brown's cases. Many of the patients became infected with two, three, or even four different types in succession. At the time of discharge, after an average stay in hospital of 33 days, 39 patients were still infected. The primary strain disappeared, on an average, after 18 days; the cross-infecting strain persisted for a time varying from a few days to a few weeks.

The result of such cross-infections varies according to circumstances. The infection may remain latent; it may give rise to a transient secondary fever, accompanied or followed by complications such as coryza, tonsillitis, adenitis, or otitis; or it may be responsible for a completely fresh attack of scarlet fever. Allison and Brown (1937) found that the majority of complications occurring in multiple bed wards after the end of the second week in hospital were due to cross-infection. Our own experience would suggest caution in accepting this finding as necessarily true of all hospitals, or of the same hospital at all times.

Though a high proportion of scarlet fever patients are still *infected* on discharge from hospital, it is difficult to say how many of them are actually *infective*. The return case rate does not as a rule appear to exceed 2 per cent., though slightly higher figures are sometimes quoted. Brown and Allison (1935) found that heavy carriers were more likely to prove infective than light carriers, and that patients discharged during the 5th, 6th and 7th weeks were more infective than those discharged earlier. This observation is interpreted as suggesting that the primary infecting strain loses most of its infectivity by the 4th week, and that the cross-infecting strain is still infective at the time of the patient's discharge.

Epidemiological evidence indicates that the great majority of patients infected with streptococci become relatively harmless to their fellows within 3 or 4 weeks. As Griffith (1934) suggests, this may be due to a process of antigenic degradation accompanied by loss of virulence occurring in the throat of a person who has become immune to the particular type of streptococcus with which he is infected. On the other hand, numerous—mainly unpublished—observations have been made showing that an occasional convalescent carrier may remain infective for weeks or even months, as evidenced by his ability to give rise to an outbreak of sore throat or scarlet fever due to the same type of streptococcus in an institution to which he is admitted (Griffith 1934, Moore 1943, Boissard and Fry 1944, Coburn 1944*a*). No bacteriological test is available to distinguish between infective and non-infective strains of *Str. pyogenes*, but it is usually found that persistently infective carriers have some abnormality of the upper respiratory tract, such as a discharge from the nose or ears or enlarged unhealthy tonsils.

The extent to which infection spreads among a population has been studied in institutions (see Griffith 1934, Report 1938, Straker *et al.* 1939, de Waal 1941) and other communities (Schwentker 1943, Schwentker *et al.* 1943*a*, *b*). The attack

rate is subject to considerable variation, seldom, however, in this country exceeding 10 per cent. and often being very much lower. Though there may be a parallelism between the carrier rate and the attack rate, numerous exceptions occur. From institutional studies we have learned that there may be few cases even in the presence of a high carrier rate, and many cases when the carrier rate is low. Hamburger (1944) has had the same experience. This does not seem to be determined wholly or even mainly by the previous streptococcal experience of the children; and as Griffith's studies show, immunity in any case is more or less type-specific. The evidence is far more in favour of Griffith's conception of the existence of strains possessing varying degrees of infectivity and invasiveness (see Stocks 1941). An "epidemic" strain, endowed with both these qualities in a high degree, is liable, when introduced into a given population, to cause a relatively high morbidity, whereas other strains less liberally endowed in these respects will be responsible for merely a few sporadic or "dropping" cases. Analogy with experience of the meningococcus is very close. An additional factor that may exert a considerable effect on the extent and severity of scarlet fever is the coincidence of other respiratory disease, such as measles or influenza (see Gordon 1939). According to Stebbins, Ingraham and Reed (1937), who made a very careful study of milk-borne streptococcal outbreaks in New York State, children under 15 years of age are more infective than older children or adults. The secondary attack rate among household contacts was nearly twice as high when the infecting case was a patient less than 15 years old—13·7 as opposed to 7·3 per cent. The same workers quote figures of 20·0 and 5·3 per cent. for children under and over 15 years respectively in a non-milk-borne outbreak in the same State. Gordon (1940) states that children under 5 years are responsible for about eleven times as many secondary cases as are patients aged 15 years or older. Coburn and Pauli (1941) likewise found that children were much more infective than adults.

Mode of Spread of Infection.

Spread of scarlatinal infection is believed to be usually due to droplets or droplet nuclei, the source of infection being a case of tonsillitis with or without a rash, or a convalescent or normal carrier. Hæmolytic streptococci have been isolated by some observers from the desquamated scales of scarlet fever patients (see Allison and Gunn 1932); but it is unlikely that this is a frequent source of natural infection. Far more important—at least in hospitals—is aerial spread by infected dust. Numerous workers have now demonstrated the presence of hæmolytic streptococci in the *dust* of wards or dormitories housing patients suffering from scarlet fever, tonsillitis, or other streptococcal infection (White 1936, Cruickshank and Godber 1939, Stalker *et al.* 1942, Walter and Hucker 1942, Edward 1944; see also Chapter 91). These organisms may remain alive and apparently virulent for several weeks, especially if protected from light; and there is reason for believing that cross-infection in hospitals is often due to dust derived from the floor, the bedclothes, or the patient's handkerchief.

Apart from personal contact and dust, an important mode of spread of scarlet fever is by *milk*. Since the classical Hendon outbreak of 1885, there have been numerous epidemics, both in Europe and America, in which the epidemiological evidence incriminating milk has been ovewhelming. Of more recent years, this has been supplemented by bacteriological proof, consisting of the demonstration of the same type of infecting organism in the milk as in the throat of the patients.

Though it was believed at one time that the usual source of infection of the milk was the cough spray or ear discharge (see Henningsen and Ernst 1939) of a worker on the farm or in the dairy, the evidence is now pointing more and more in favour of infection of the udder of the cow itself. Most cases of streptococcal mastitis are due to *Str. agalactiæ*; but in occasional cases an organism indistinguishable from *Str. pyogenes* is responsible. In such instances the milk may contain enormous numbers of streptococci that prove highly infective for man. Milk that is contaminated directly from a human case or carrier is liable to be less infective, because *Str. pyogenes* does not usually multiply to any considerable extent in milk at room temperature (Pullinger and Kemp 1937). Several outbreaks have now been recorded in which infection has been traced to the diseased udder of a cow, and in some a human carrier has been found from whom the cow presumably derived its infection (see Jones and Little 1928*a*, *b*, Camps and Wood 1936, Watson 1937, Bendixen and Minett 1938, Henningsen and Ernst 1938, Douglas *et al.* 1941, Dublin *et al.* 1943). Stebbins, Ingraham and Reed (1937) were able to trace infection in 6 out of 7 streptococcal outbreaks of sore throat to mastitis in one of the animals supplying the milk. In the remaining outbreak the milk had been infected from a human source, having been bottled and capped by a worker suffering from an acute sore throat (see also Dublin *et al.* 1943). It may be noted that in these outbreaks, three of which were classified as scarlet fever and four as septic sore throat, patients who gave a history of a previous attack of scarlet fever suffered as severely as those with no such history. The case mortality rates and the incidence of complications were the same in both groups. The only difference was in the frequency of appearance of a rash, the incidence being less than 30 per cent. in the former, and nearly 65 per cent. in the latter group.

The history of scarlet fever provides an excellent example of an infective disease which has exhibited variations in its behaviour during the past half-century. We have already noted the mildness of the type of infection now prevalent; but this mildness is a relatively recent development. In 1861–5 the death rate from scarlet fever, in England and Wales, was 982 per million living; twenty years later it had dropped to half this value; and in the quinquennium 1921–5 it had fallen to 29 per million. This decrease in mortality has, during recent years, been associated with a shift in the age incidence of the disease. The morbidity in the 5–10 age group has increased at the expense of that in the 0–5 age group; while the maximum mortality now tends to fall on older children and young adults.

Prophylaxis

In a disease like scarlet fever, in which the carriers among the general population largely outnumber the cases, we should not expect isolation in fever hospitals to exert any appreciable effect on the frequency of the disease among the population at large; and we may note that Woods (1928), in a statistical study of the incidence of scarlet fever in various districts in this country over a period of 25 years, could find no evidence that isolation had any effect on the prevalence of the disease.

So far as the patient himself is concerned, it must be remembered that cross-infections in hospitals are very common, and that they may be responsible for complications and for increasing the duration of treatment. Their incidence may be greatly reduced by nursing the patients in cubicle blocks, or in wards reserved for patients infected with the same serological type of organism (Allison and Brown

1937, Cooke and Neisser 1937, Peters *et al.* 1942), though the administrative difficulties of the latter method are often considerable. A further measure that promises success is the treatment of floors and bed clothes with dust-laying oils (see Wright *et al.* 1944, Harwood *et al.* 1944, Report 1944, and Chapter 91). In order to diminish the risk of cross-infections, it is wise, if possible, to isolate the patient at home, or, if admission to a hospital is advisable, to restrict the stay to the minimum period necessary—usually 2 to 3 weeks (see Gordon 1940).

Though sulphonamide treatment of the disease itself, apart from complications, seems to be of little value, it is possible that low prophylactic may prove useful in appropriate circumstances. Watson and his colleagues 1943 record apparent success from the daily administration of 1 gm. of sulphadiazine to contacts during an epidemic of scarlet fever in a large naval station. Our own experience has been less favourable (Mollaret and Wilson 1947).

Outbreaks of scarlet fever in schools and other institutions are often very difficult to deal with. Segregation of heavily infected carriers and the passive immunization of contacts may at times be called for, but our control over this disease cannot be compared as yet with the ease with which we can bring outbreaks of diphtheria to a sudden close. Particular care should be taken before admitting convalescents to an institution. Though the majority of convalescent carriers appear to become non-infective within 3 or 4 weeks, occasional carriers may remain infective for much longer and be responsible for introducing the disease into the community. Search should be made in all convalescents for abnormality of the upper respiratory passages, and any child who is found to be a carrier of *Str. pyogenes* and to have rhinorrhœa, otorrhœa, or enlarged unhealthy tonsils, should be regarded as potentially dangerous and treated accordingly. Nasal infections often yield to sulphapyridine snuff (Boissard and Fry 1942, 1944).

Active Immunization against Scarlet Fever and Passive Protection with Serum.

The reagent used for active immunization is a broth filtrate of a culture of a strain of *Str. pyogenes* containing the erythrogenic toxin. This toxin is not converted into toxoid before use. Though some observers (see Veldee 1931, 1933) have reported favourably on the immunizing value of a formol toxoid, similar to that employed in diphtheria immunization, most observers have found such preparations to be relatively inactive (Okell 1932, Dick and Dick 1934). Moreover, unlike diphtheria toxin, the erythrogenic toxin is relatively harmless. The use of the unmodified toxin for immunization rarely leads to any severe local reactions though troublesome reactions are more numerous than in diphtheria immunization. Generalized reactions of the "miniature scarlet fever" type can be avoided by decreasing the initial dose, and working it up very gradually, in those subjects whose reaction to the Dick test shows them to be unusually susceptible.

From our knowledge of the pathogenesis of the disease we should expect that immunization with the erythrogenic toxin would produce an effective immunity against that part of the total scarlatinal syndrome to which it gives rise—the characteristic rash and probably many of the other toxic symptoms. We should not expect any antibacterial immunity. There is no evidence to suggest that the filtrate injected contains an effective dose of bacterial antigen; and, even if it did, the resulting increase in resistance would only be operative against a single antigenic type of hæmolytic streptococcus, while a variety of types may give rise to typical scarlet fever. There is therefore no strong reason to suppose that this

method of immunization will protect against the local proliferation of hæmolytic streptococci in the throat, or their possible spread to other regions; though it might have such an effect if the erythrogenic toxin played a part in damaging the tissues and allowing the streptococci to proliferate.

Extensive field trials have been made and the results are in general accord with expectation, though it seems doubtful whether the immunity induced is as effective as that obtained in immunization against diphtheria.

It would appear (Benson 1928) that the total dose of toxin required to induce immunity may be relatively large—20,000 skin test doses. For persons who give mildly positive Dick reactions 1,000 skin test doses usually form a safe initial dose, but in those who give a vivid and extensive Dick reaction it is wiser to commence with the injection of 500 skin test doses, or even less. Injections may then be given at weekly intervals, increasing the dose 2 to 5 times at each successive injection until a total of 20,000 skin test doses has been given in the course of 3 to 4 weeks. Dick and Dick (1929) have recommended even larger doses, commencing with 500 and working up to 80,000 to 100,000 skin test doses. Kolchin and Klein (1941), immunizing a small series of nurses, found it necessary in order to bring about conversion from the Dick-positive to the Dick-negative state, to use from 9 to 19 injections totalling from 20,000 to 338,000 skin test doses (see also Veldee 1937, 1938, Toomey 1941).

The evidence with regard to the proportion of susceptibles that can be rendered Dick-immune by active immunization is less extensive and less concordant than that available in the case of antidiphtheria immunization. It has been the experience of many observers that the proportion of cases in which a Dick-positive is converted into a Dick-negative reactor is lower and the relapse rate higher, than the corresponding figures for the change in the Schick reaction induced by diphtheria immunization (see Heller 1927, Toyoda *et al.* 1929, Okell 1932).

The records of the incidence of scarlet fever in the immunized and non-immunized are as yet too few to afford a safe basis for any final conclusion as to the effectiveness of the procedure under different conditions; but they clearly suggest that it has considerable value. Toyoda and others (1929) record a scarlet fever incidence of 1 per 1,000 among fully immunized persons, 18·1 per 1,000 among those partially immunized, and 62·5 per 1,000 among non-immunized controls. Similar favourable results in various communities have been reported by Platou (1934) Henry (1935), Krumbiegel (1938), Little (1939), Wartiovaara (1939) and Veldee and his colleagues (1941); the scarlet-fever incidence rates in the non-immunized vary from 4 to 14 times as great as those in the immunized. As in diphtheria immunization, the most significant results have been reported from fever hospitals or other institutions in which the risk of contracting scarlet fever is unusually high.

Benson (1928) records the effect of Dick-testing and immunization on the incidence of scarlet fever among the nursing staff of the Edinburgh City Fever Hospital. From 1919 to 1925, the incidence of scarlet fever fluctuated between 4·82 and 10·27 per cent. Immunization was started in October 1925; in 1926 the incidence was 3·52 per cent. and in 1927 it was 0·67 per cent. The figures for 1928–31, as given in the Annual Reports of the Medical Officer of Health for Edinburgh, show that the very low incidence of scarlet fever has been maintained.

Dick and Dick (1929), summarizing their records, state that among 11,584 immunized persons in institutions where scarlet fever was epidemic, no case of scarlet fever occurred, and that of 1,191 nurses or interns immunized before commencing work in fever hospitals none contracted the disease.

In a group of nurses aged 18–25 years, Cruickshank (1936) found the percentage incidence of scarlet fever to be 0·0 in the naturally Dick-negative, 2·8 in nurses Dick-negative by immunization, 15·0 in nurses who were positive after immunization, and 25·0 in nurses who were originally positive but were not immunized.

It may be noted that the experience of active immunization in man is in general accord with the view that the protection afforded is less effective against the invasive than against the toxæmic effects of haemolytic streptococcal infection. Thus Kinloch and others (1927) note an increase in the frequency of streptococcal tonsillitis among the nursing staff of a fever hospital after its immunization, suggesting that the typical scarlatinal syndrome had been replaced by an infection confined to the throat. Harries (1927) notes a similar fact; he records a slight diminution in the incidence of scarlet fever among the staff after immunization; but the diminution was by no means so striking as the much greater decrease in the incidence of scarlet fever among the staff over the same period (see also Place 1928).

In practice, owing partly to the difficulty of giving five injections, partly to the severe clinical reactions that follow in some subjects, and partly to the absence of protection conferred against infection as distinct from toxæmia, the use of active immunization is very limited. Its chief value is for nurses in fever hospitals, for some children's institutions, and for countries in which the death rate from toxic scarlet fever is high.

Inoculation of contacts with antitoxic serum affords passive protection for 2 to 3 weeks, and may be used in sick or debilitated children, or for other special reasons, when the risk of repeated exposure is negligible.

The Treatment of Scarlet Fever.

Antitoxic Sera.—Clearly, the demonstration that a high-titre antitoxic serum can be obtained by immunizing a horse against erythrogenic toxin raises the question of the value of such antisera in the treatment of the disease in man.

It seems reasonable to suppose that a specific antitoxic serum will exert an effect on those manifestations of scarlet fever that are directly toxic in origin. We might expect a saving of life in severe toxæmic cases, a diminution in the rash and a more rapid subsidence of the fever in the milder type of infection. Whether the serum exerts an indirect effect on the bacterial invasion, by lifting the burden of toxæmia from the body and thereby improving its antibacterial defences, it is difficult to say.

Figures in regard to death or recovery are not easy to obtain because the prevalent type of scarlet fever is mild and the case fatality low. Such figures as are available support the view that there is a significant saving of life in severe toxæmic cases when these occur.

Toyoda and his colleagues (1929) record 8 deaths in 33 serum-treated cases of severe scarlet fever against 15 deaths in 33 untreated cases of the same order of severity; and, so far as data with regard to death or recovery are forthcoming, other records are in general conformity with these observations. Thus Mersol (1929), during an epidemic of scarlet fever in Jugoslavia associated with a high case-fatality rate, records 4 deaths among 117 serum-treated patients (3·4 per cent.) as against 84 deaths among 377 untreated patients (22·3 per cent.).

In regard to the diminution in severity of the rash, the subsidence of fever, and the general alleviation of toxic symptoms, the consensus of opinion clearly supports the view that antiscarlatinal serum has a beneficial effect (see Benson and Maciver 1926, Harries 1927, Discussion 1927, Kinloch and others 1927, Husler 1927, Lenthe 1927, Eley 1928, Scott 1928, Bröcker 1928, Friedemann and Deicher 1928, Toyoda and others 1929, Veldee and others 1931). It should be noted that Veldee and his collaborators, almost alone among those who have reported on this point, failed to demonstrate any significant effect on the temperature although there was a striking effect on the rash.

[illegible] above suggest that anti- [illegible] the frequency of complications; but there are a [illegible]

Hewitt 1927 [illegible] with antitoxin, and 1,190 untreated controls. [illegible] admissions in order to obtain a random [illegible] 15 cases with complications, among the [illegible] not entirely accounted for by the different [illegible] in the treated. Veldee and others 1931 [illegible] 112 serum-treated patients and 84 controls, the allocation [illegible] serum groups again being by alternation of admission. The difference in the frequency of complications was striking, that in the control group [illegible] cervical adenitis, 19·5 per cent. as against 0·4 per cent.: otitis [illegible] 17·4 per cent. as against 7·3 per cent.: suppurative otitis, 7·3 per cent. as against 3·6 per cent. and so on. Those complications that are probably due to toxæmic rather than invasive processes showed the same wide differences—nephritis, 12·2 per cent. in the controls as against 0·0 per cent. in the treated: arthritis, 6·1 per cent. as against 0·0 per cent. Burke 1933, 1936, also has produced striking evidence of the efficacy of antitoxin in reducing not only the death rate, but the incidence of complications. From a large, carefully controlled series of observations he concludes that the intravenous or intraperitoneal administration of 10–20 ml. of antitoxin in a single dose reduces the incidence of otitis media by one-third and of nephritis by one-seventh. It is noteworthy that by intramuscular antitoxin he could produce no statistically significant reduction in the incidence of complications (but see Ronaldson 1937). Neukirch, Zahle and Baumgarten (1943), giving antitoxin early in the disease to all cases irrespective of the degree of toxæmia, reduced the complication rates to a small extent as compared with control cases, but found that the benefit was outweighed by serum sickness, which occurred in 64 per cent. of the patients. In this connection it should be noted that Chandler and Hartshorn (1933) observed in four scarlet fever patients treated with antitoxin the coincidence of a streptococcal bacteræmia with the onset of serum sickness, and suggested that the serum sickness might have contributed to the exacerbation of the infection (see also Burn, Chandler and Hartshorn 1934).

Summing up, we may say that there is clear evidence that antitoxic serum has a significant therapeutic effect on the toxic manifestations of scarlet fever, and suggestive evidence that it may—contrary to our *a priori* expectations—lessen the frequency of those complications that are due to the invasive action of the streptococci.

In practice, it is advisable to give antitoxic serum—preferably of the refined protein-digested type—to patients nursed in an open ward. In mild cases it should be injected intramuscularly, in severe cases intravenously. Though it may protect to some extent against complications, it has no effect once these have occurred. It may be noted that some workers prefer convalescent serum in the treatment of scarlet fever, on the ground that it probably has antibacterial as well as antitoxic properties (see Gordon 1933, Platou, Dwan and Hoyt 1941).

The Standardization of Scarlatinal Antitoxin.—As in the case of all other antisera, the standard of reference is an arbitrarily selected sample of serum. It happens that the unit of this serum was defined as 10 times the amount that, in the skin-neutralization test, will protect against the "test dose" of toxin; while the "test dose" is defined as five "skin test doses," and a "skin test dose" as the least amount of toxin that, in a majority of susceptible persons, gives rise to a local skin reaction of not less than 10 mm. in diameter (see Dyer 1928). But there is nothing exact or binding about this definition. The Standard American serum was adopted

by the Standardization Committee of the [illegible] national Standard and Unit [illegible]

The great difficulty, which may [illegible] of any suitable experimental animal [illegible] titrated.

Kirkbride and Wheeler 1926 [illegible] and Ando and Kuranchi 1930 in white pigs; [illegible] generally confirmed.

Until recently the most hopeful approach [illegible] by Parish and Okell (1927). Rabbits are [illegible] the serum under test, and, 4 to 6 hours later, with [illegible] virulent strain of a hæmolytic streptococcus. The [illegible] the acute septicæmic death that occurs in control. [illegible] the antitoxin die eventually of a subacute streptococcal [illegible], pericarditis, etc.; but death seldom occurs before the 6th day, and this is [illegible] end-point (see also O'Brien, Okell and Parish 1929).

In 1930, however, Fraser and Plummer described a method of titrating [illegible] and antitoxin in the skin of Chinchilla rabbits; and further experience with this test suggests that, with certain modifications, it gives results as accurate as those obtained in the human skin, or by the method of Parish and Okell (see Kolmer *et al.* 1933, Plummer 1934, Buttle and Lowdon 1935). Veldee (1932) and Malcolm and Wyman (1937) developed a similar test, using the ear of the rabbit. Many attempts have been made to titrate the erythrotoxin by a constant-antigen flocculation method analogous to the Ramon titration of diphtheria toxin. More recently, successful *in vitro* titrations of erythrogenic toxin, tallying with those in man and animals, have been made, using solutions of toxin containing over 50,000 skin test doses (Rane and Wyman 1937, Bunney and Kneipel 1941, Hottle and Pappenheimer 1941, Proom 1941).

Convalescent Serum.—The use of convalescent serum in the treatment of scarlet fever has the obvious advantage that to a large extent serum reactions are avoided (see, for example, Fox 1937). Large-scale tests of pooled convalescent serum indicate that it may be at least as effective as antitoxin in reducing both mortality and complication rates (Fox and Hardgrove 1937). Top and Young (1939, 1941), who recommend its use in the septic forms of scarlet fever, found that a pooled serum containing 60 antitoxic units per ml. was as effective as a horse antitoxin containing 6000 units per ml., and concluded that convalescent serum owed its efficacy to antibacterial as well as to antitoxic antibodies. Moore and Thalhimer (1939) tested convalescent sera for antitoxic and antibacterial activity. The antitoxin content averaged 3·3 units per ml. The bactericidal power, measured on four antigenically different strains of Group A streptococci, was, when present, to some extent strain-specific. Platou, Dwan and Hoyt (1941) tested the bactericidal power of whole blood, and demonstrated that convalescent bloods were more bactericidal than normal bloods, and that the effect was independent of antitoxic immunity. These results support the suggestion of an antibacterial action. If the action is type-specific, then only pooled polyvalent sera, or type-specific convalescent sera, will be efficacious in the treatment of scarlet fever.

Chemotherapy.—In the treatment of scarlet fever cases sulphonamides appear to be of little value except for dealing with complications. It is true that the routine use in all patients has been recommended by some workers (Neukirch *et al.* 1943); but there is little evidence to show that they influence the course of the disease, and if protection is to be afforded against both early and late complications

average resistance of the various communities affected can have differed so widely as to account for the marked difference in clinical manifestations.

We should not, in such a conception of *Str. pyogenes* infections, interpret the term "toxigenicity" too literally. It is very probable that invasiveness and perhaps the capacity to multiply freely in the tissues at the original foci of infection are at least equally involved. The determining factor will be the ability of any given strain to produce toxin plentifully and rapidly in the tissues, and this involves many factors that are not concerned in the production of toxin by an organism growing under optimal cultural conditions in the laboratory. We may recall the fact that the undoubted differences in virulence between the *gravis*, *intermedius* and *mitis* types of the diphtheria bacillus are not closely paralleled by differences in toxigenicity as judged by growth *in vitro*.

It may, perhaps, be added that the studies of Griffith and his colleagues on epidemics of hæmolytic streptococcal infection, particularly in schools, supply some of the strongest evidence in support of the view that there exist, within any given pathogenic bacterial species, particular "epidemic strains" endowed with a heightened power of spreading naturally among the population at risk. Reference to Chapter 56 will show that this view is also supported by the results obtained in experimental epidemics.

Mention has already been made under scarlet fever of the occasional prolonged infectivity of convalescent carriers having enlarged unhealthy tonsils. In some of these, throat swabs may fail to detect hæmolytic streptococci. *Str. pyogenes* can usually be isolated from about 50 per cent. of excised tonsils (Elliott 1939, Keogh *et al.* 1939*b*, Macdonald *et al.* 1940). Rantz (1941) records figures of 32·7 per cent. from the tonsils after removal, but of only 16·4 per cent. from throat swabs before tonsillectomy.

In the prevention of tonsillitis *chemoprophylaxis* with the sulphonamide group of drugs is now being tried. Too little information is yet available on which to assess the value of this method, but large-scale observations in the United States army and navy suggest that streptococcal infections of the upper respiratory tract, together with complications such as otitis media, may be considerably reduced in incidence by the daily administration of 1 gm. of sulphadiazine (Holbrook 1944, Coburn 1944*b*). The conditions under which these trials were made, however, were not altogether satisfactory, and the experience so far gained in this country is not reassuring. Reports on the use of sulphonamides in the treatment of acute tonsillitis are conflicting, but no striking benefit seems to result from their administration. The response to penicillin, both clinically and bacteriologically, is said to be rapid, but relapse is common unless treatment is maintained for at least 6 days; even so, complications cannot be entirely prevented (Plummer *et al.* 1945).

Erysipelas

This classical example of acute streptococcal infection may, for our present purposes, be shortly dismissed. As an experimental disease it has been discussed in some detail in Chapter 53, in connection with the general problem of local immunity. There is evidence (see for example, Keefer and Spink 1936) that in some cases the attack is preceded by an infection of the throat or upper respiratory tract, and it is noteworthy that in 10 of 37 patients with erysipelas, de Waal (1941) found the same type of *Str. pyogenes* in the skin lesion and in the throat; but it is not clear whether the streptococcus, if it is the cause of the erysipelas, reaches

... usually and sets up 'traumatic erysipelas, or by an internal route, and ... an apparently "idiopathic" erysipelas.

The interest of erysipelas, in relation to the question of streptococcal immunity in general and scarlatinal immunity in particular, lies in the well-attested fact that a single attack of the disease confers no protection against subsequent infection; indeed, some persons appear to possess a marked predisposition to the disease, and suffer from many attacks throughout their lives—usually in the same skin area. The striking contrast in this respect, between erysipelas and scarlet fever, suggests an essential difference in the mechanism of infection and resistance, and it would seem reasonable to accept, as a purely provisional hypothesis, the view that the antitoxic immunity, which is the essential factor in resistance to scarlet fever, is relatively ineffective as a protection against the invasion of the skin by *Str. pyogenes*, which is the essential feature of erysipelas.

This does not of course involve the view that the erythrogenic toxin plays no part in erysipelas: indeed, there are several reasons for believing that it may be responsible for certain features of the disease (see Okell 1932). It means only that a degree of antitoxic immunity that will protect against scarlet fever will not protect against an erysipelatous infection of the skin.

Puerperal Fever

In discussing the problem of puerperal infection it has become customary, among those who are concerned with this problem from the clinical aspect, to adopt some arbitrary standard of pyrexia and to include in the febrile category all cases which show a rise in temperature beyond this limit of duration. Such a classification, however useful it may be from the clinical point of view, can have little relation to aetiological factors; and it is probable that the milder degrees of puerperal pyrexia include a miscellany of bacterial infections, which cannot, at the moment, be classified on any bacteriological basis.

As a cause of puerperal septicæmia, however, there can be no doubt that the hæmolytic streptococcus holds a pre-eminent position (London and N. England Committee 1925, Fitzgibbon and Bigger 1925, Colebrook, L., 1926, Kinloch *et al.* 1928, Colebrook, Dora C., 1935, and others). Colebrook, D. C. (1935), for instance, in reviewing the results recorded by various workers, notes that among the fatal cases of puerperal fever the proportion due to infection with hæmolytic streptococci varied from 68 to 96 per cent. The majority of these streptococci belong to Group A, though a small proportion of puerperal infections are associated with Groups B, C, D and G, and very rarely, some of the remaining groups (Lancefield and Hare 1935, Gardner 1939, Chatterjee and Mitter 1941). Infection by hæmolytic streptococci other than Group A is not usually severe, though among these milder infections a number of authors have noted severe and sometimes fatal cases of septicæmia, often associated with infective endocarditis, following puerperal or post-abortion infection by streptococci of Groups B, G and rarely C (Colebrook and Purdie 1937, Fry 1938, Macdonald 1939, Hill and Butler 1940, Rosenthal and Stone 1940, Ramsay and Gillespie 1941).

Since the severe infections are due to *Str. pyogenes*, we must consider the part played by the erythrogenic toxin in the genesis of the infection, and by antitoxic immunity in resistance to it. If an antitoxic immunity of this type conferred any increased resistance to puerperal infection with hæmolytic streptococci we might reasonably hope to induce a significant decrease in maternal mortality by antitoxic immunization, carried out during

pregnancy. In view, however, of our general knowledge of the role of [illegible] antibacterial immunity, we should [illegible] to afford any significant protection against an invasive [illegible] leading to septicæmia; and the facts are in [illegible] Colebrook and others (1930), Stent (1930) and Bird [illegible] that severe puerperal infections are just as common in [illegible] as in those who react positively. Moreover, Kenny and Colebrook (1927) found that none of 19 women who were Dick-positive in the early stages of pregnancy [illegible] Dick-negative as a result of that infection. Clearly [illegible] genic toxin from the lesions in the genital tract. Very [illegible] panied by a scarlatiniform rash, and it is noteworthy that in [illegible], Kenny and Colebrook found a powerful erythrogenic toxin in the exudate from the genital tract.

It does not of course follow that the other toxins produced by haemolytic streptococci, the hæmolysin, leucocidin and so on (see Chapter 24) and the corresponding antibodies, play no significant part in puerperal infection; but it may be stated that the balance of evidence at present available is in favour of the view that the decisive factor in attack is the invasiveness of the organism, and that defence depends, in the main, on an antibacterial immunity that is type-specific. We shall return to this question in relation to prophylaxis and treatment.

Sources of Infection.—The most important question from this point of view is as to whether the majority of puerperal infections with haemolytic streptococci are intrinsic or extrinsic. These terms are usually employed to differentiate between those cases in which the hæmolytic streptococcus is present in the female genital passages before labour, and those in which it is introduced from without during labour or the puerperium. The problem at issue therefore narrows down to the presence or absence of the hæmolytic streptococcus in the cervix or vagina during the later periods of pregnancy. If it is frequently present under normal conditions, we should lay particular stress on host susceptibility, and on the extent of tissue injury during labour, as factors determining the occurrence of puerperal fever: if it is usually absent, we should regard infection from without as the essential factor, and stress the vital importance of asepsis and antisepsis in handling maternity cases.

Fromme (1908) found streptococci in the vaginal secretions of 46 of 136 pregnant women, but none was hæmolytic. On the other hand he cultivated hæmolytic streptococci from almost every case of severe infection during the puerperium. Lüdke and Polano (1909) found no hæmolytic streptococci in 30 cases during a normal puerperium. Kanter and Pilot (1924) examined 96 cases during pregnancy, and found hæmolytic streptococci in one case only. Lockhart (1925) in a careful study of 100 cases, during which swabs were taken before and after labour, found hæmolytic streptococci in one case only before labour occurred, though streptococci of other types were frequent. One other case showed the presence of this organism in a swab taken immediately after labour; and in four other cases, in each of which a single swab before labour and two taken in the first few days of the puerperium were negative, a copious growth of hæmolytic streptococci was obtained from a third swab, taken immediately the temperature began to rise. Taylor and Wright (1930) isolated 32 strains of hæmolytic streptococci from vaginal swabs taken from 1,123 pregnant women immediately after labour; only 18 (1·6 per cent.) could be classified as *Str. pyogenes* on the basis of fermentation reactions.

It may then be stated with some confidence that hæmolytic streptococci are very infrequent in the genital passages during pregnancy, being present in something under 3 per cent. of cases. In the early days of the puerperium they are rather more frequent, being found in about 5 to 6 per cent. of all cases (see Hare

... the observation of these cases showed that those women who were harbouring hæmolytic streptococci in their genital passages before labour were particularly liable to suffer from puerperal fever the hypothesis of intrinsic infection would receive strong support; but this is not so. There is general agreement that the great majority of them pass through a perfectly normal puerperium and that this is also true of a high proportion of those women from whose genital passages hæmolytic streptococci are isolated during the puerperium itself. The reason for the harmlessness of these streptococci is clear. Few of them are *Str. pyogenes.*

Hare and Colebrook (1934), for example, found only 2 of 52 strains to be *Str. pyogenes* according to fermentation reactions; about half gave the reactions of *Str. agalactiæ* (Chapter 24). Lancefield and Hare (1935) found one Group A strain among 65 vaginal strains isolated during an afebrile puerperium, and none among 13 strains isolated before labour.

It may be added that Colebrook, Maxted and Johns (1935) failed to detect *Str. pyogenes* on the perineal or peri-anal skin of 160 women attending an antenatal department, and that Hare and Maxted (1935) failed to isolate *Str. pyogenes* from the fæces of 100 normal women during the first stage of labour.

There can then be very little doubt that *Str. pyogenes* is not a very frequent inhabitant of the normal genital passages either before or after labour. In Lancefield and Hare's series, for instance, the 13 strains of hæmolytic streptococci isolated before labour were obtained as the result of swabbing 855 women, and none of them was *Str. pyogenes.* The hypothesis of intrinsic puerperal infection receives very little support from such studies as these.

If we accept the view that infection is usually extrinsic in origin it is clearly of importance to determine its source. An obvious working hypothesis is that the hæmolytic streptococci are derived from those in attendance on the patient, or at least in her immediate entourage. Until recent years it was extremely difficult to test this hypothesis by observation. The normal carrier rates quoted earlier in this chapter will show that, if 5 to 10 persons have come into contact with a patient during labour in the early days of the puerperium, there is a very good chance of one or other of them carrying a hæmolytic streptococcus in the throat. It would be quite unjustifiable to assume on such a basis that the carrier in question had infected any particular case of puerperal fever.

The division of *Str. pyogenes* into 40 or more distinct serological types has, however, entirely altered the position.

Taking an average figure of 6 per cent. for the normal carrier rate of *Str. pyogenes* in the throat (see above), we should expect about one person in seventeen who came into contact with a parturient woman to be a possible source of danger. But if this person may be harbouring any one of the 40 or more different types, and the patient may also be suffering from infection with any one of these types, the odds against the type isolated from the contact being identical with the type isolated from the patient become very high indeed, unless the patient has derived her infection from the contact, or *vice versa.* We cannot multiply 40 by 40 to get the exact odds, because that would assume that the carrier rate for each type of streptococcus was equal, which is almost certainly not the case; but the odds are certainly high enough for an established identity of type to be significant.

Two series of observations are on record in which this method has been applied, using the technique of agglutination and agglutinin absorption for the antigenic

analysis of the strains isolated. Smith (1931, 1933) was able to show that, in 39 of 49 cases of puerperal fever due to *Str. pyogenes* a strain identical with that derived from the patient was also found in the throat or nose of the midwife, or doctor, or some other person in attendance on the patient or, more rarely, in the throat or nose of the patient herself. In 8 cases only the latter event occurred in 8 cases as opposed to 31 cases in which it was the probable source of infection.

Dora Colebrook (1935) has recorded a very careful and detailed study on similar lines, involving the examination of 60 cases of puerperal fever and their contacts. In 38·1 per cent. of these cases a haemolytic streptococcus antigenically identical with that derived from the blood or uterine discharges of the patient was also isolated from the patient's throat or nose. In 57·1 per cent. of the cases a strain antigenically identical with that causing the infection in the patient was isolated from the throat or nose of one or more persons in contact with the patient. In a proportion of these cases (22·2 per cent.) both the patient and a contact were carrying a strain identical with the infecting strain. Thus in 73 per cent. of the cases a strain identical with the infecting strain was isolated either from a contact or from a patient or from both.

We may conclude that, in a very high proportion of cases, the most probable source of uterine infection with *Str. pyogenes* during labour and the puerperium is from the throat or nose of some person in contact with the patient, or from the throat or nose of the patient herself. We must regard this transfer from outside sources to the genital passages as probable rather than certain, because we cannot exclude the possibility that transfer sometimes occurs in the reverse direction.

In regard to the mode of transference an observation of Colebrook, Maxted and Johns (1935) is of considerable interest. They were able to isolate *Str. pyogenes* from the skin of the hand in 7 of 181 normal persons (3·8 per cent.). Of 248 normal persons Hare (1941) found only one (0·4 per cent.) to be carrying Group A streptococci, and our colleague, Dr. R. E. O. Williams, only 4 (0·5 per cent.) of 875 persons. Taking the normal carrier rate in the throat as about 6 per cent., and regarding this as the most probable source of hæmolytic streptococci found on the hands, an obvious alternative is provided to direct droplet infection.

Prophylaxis.—If an effective method of active immunization were available the conditions for applying it would be peculiarly favourable, since we should be protecting against a risk occurring at a predictable time, with several months at our disposal during which immunity could be induced. Unfortunately no such method is at our disposal, nor is the immediate outlook hopeful. An antitoxic immunity, if effective, in this disease, would confer protection against all types of *Str. pyogenes*, since all form the same toxins. As we have seen, however, there is no evidence that such an immunity confers resistance against the invasive powers of the streptococcus. Such antibacterial immunity as we can induce by the injection of a bacterial vaccine, as Stamp (1936) has shown experimentally, will be type-specific, and it would therefore be necessary to include in our vaccine all the antigenic types that are responsible for puerperal infection. When we know more than we do now in regard to the actual frequency of these various types in puerperal fever it may be possible to prepare a vaccine that will protect against the types that are most often met with; but our present data contain no indications that any particular type, or any small group of types, will be found to cover a large proportion of cases.

In the absence of an effective measure of active immunization we are forced to rely, in the main, on methods designed to prevent *Str. pyogenes* gaining access to the genital passages of parturient women by droplets, dust-particles (see White 1936) and by the numerous possible methods of direct and indirect contact. Some of the lines along which such methods should be developed are sufficiently obvious from the observations outlined in this chapter. For further details see Report (1944).

Reference may be made to Chapter 54 for a description of the attempts that have been made to increase the general resistance of pregnant women by adding Vitamin A to the diet.

Serum Treatment.—It is obvious that the factors that apply in prophylactic active immunization apply also to passive immunization in an established case by the injection of a specific antiserum. We should not expect that an antiscarlatinal serum, the principal effect of which is to neutralize the erythrogenic toxin, would confer any appreciable benefit, and in fact it does not do so. Nor is there any immunological reason to suppose that any of the ordinary antistreptococcal sera on the market will prove effective. We cannot, of course, exclude the possibility that antihæmolysin, antileucocidin, or antifibrinolysin, may exert some beneficial effect; but we have no evidence that they will protect against the invasive action of *Str. pyogenes*, nor are the ordinary antisera standardized as to their content in these antibodies. If we desire an antibacterial effect we must ensure that the antibody in our serum corresponds to the surface antigen of our infecting bacterium, as we do in the treatment of lobar pneumonia (see Chapter 74); and this we cannot do. The results of adequate clinical trials are as unsatisfactory as these considerations would lead us to expect (see Colebrook, L., 1935, Kenny and Colebrook, L. 1937).

At present, then, there is no method of serum treatment in puerperal fever that is founded on a scientific basis. When we have at our disposal type-specific antibacterial sera, can ascertain the type of the infecting streptococcus, and select the corresponding antiserum for treatment, we shall be in a position to carry out clinical trials with some hope of success.

The Rôle of Anaerobic Streptococci in Puerperal Fever.—Although we are concerned in this chapter with diseases caused by hæmolytic streptococci, it will be convenient to complete our summary of the bacteriological aspects of puerperal fever by a brief discussion of the part played by other organisms.

So far as severe and fatal infections are concerned, the organisms most frequently isolated, apart from *Str. pyogenes*, are the anaerobic streptococci described in Chapter 24.

Colebrook and Hare (1933), for instance, record that, among 100 positive blood cultures from a series of unselected cases of severe puerperal infections 60 yielded *Str. pyogenes*, 38 anaerobic streptococci, and 2 both types of organism. Although the case fatality of the bacteræmic type of puerperal fever due to anaerobic streptococci is high—about 40 per cent. (Colebrook, L., 1930)—the infections are of a less fulminating type than those due to *Str. pyogenes*. It seems probable, in view of the figures given above for the observed proportions of *Str. pyogenes* infections in different samples of fatal cases of puerperal infection, that the frequency of anaerobic streptococcal infections observed by Colebrook and Hare at Queen Charlotte's Hospital will prove to be rather above the average figure; but that these organisms rank second only to *Str. pyogenes* as a bacterial cause of maternal mortality there

can be no question. A point of some interest (see Colebrook and Hare 1933) is that mixed infections are relatively common, two or more different types of anaerobic streptococci being isolated from the blood in over 50 per cent. of the cases. The occurrence of these mixed infections has also been noted by Brown (1932).

The epidemiology of these anaerobic streptococcal infections forms an interesting contrast to that of puerperal fever caused by *Str. pyogenes*. It has been shown by many observers (see Natvig 1905, Wegelius 1909, Roskowsky 1912, Sonke and Brown 1932, White 1933) that anaerobic streptococci form part of the normal flora of the female genital tract; and the observations of White (1933) suggest that this may be their principal natural habitat. In this case therefore there can be little doubt that puerperal infection is largely intrinsic. (For other references in relation to anaerobic streptococci and their rôle in puerperal fever, see Chapter 24.

Other Types of Puerperal Infection.—It remains to add that a few cases have been reported in which a fatal pyæmic infection due to *Staph. aureus* has developed during the puerperium; but these are rarities.

In regard to the less severe and fatal types of puerperal infection, which are for the most part localized to the pelvis, a variety of bacteria may be responsible. Many cases are due to a localized infection with *Bact. coli*, and these are not infrequently associated with an infection of the urinary tract. Another group of cases are due to pelvic infections with the gonococcus; and another group, especially after abortion, to infections with *Cl. welchii* (see Butler 1941).

Other Infections due to Hæmolytic Streptococci.

Cellulitis and other forms of suppuration due to *Str. pyogenes* are discussed in Chapter 67, the part played by this organism in acute respiratory infections is considered in Chapter 74, and its relation to acute rheumatism in Chapter 68.

The Use of Specific Antisera in Infections with Str. pyogenes other than Scarlet Fever and Puerperal Fever.

The considerations outlined above in the discussion of the use of antistreptococcal sera in the treatment of puerperal fever clearly apply to all other infections with *Str. pyogenes*. With the single exception of scarlet fever, and the usefulness in that disease of a serum containing the specific antitoxin that neutralizes the erythrogenic toxin, there is no immunological evidence that any of the available antisera is likely to exert a specific curative effect.

Chemotherapy in Infections with Str. pyogenes.

It has already been mentioned that sulphonamide therapy finds little place in scarlet fever apart from the treatment of complications. The same holds true of tonsillitis, though there is a suggestion that they may be of use in this disease (see Freis 1944). It should, however, be used at the first sign of invasion of other tissues than the nasopharynx. Sulphonamides, particularly sulphapyridine and sulphadiazine, are of proved value in the treatment of erysipelas, cellulitis and particularly puerperal sepsis of streptococcal origin. It is too early yet to say what part penicillin will play in future in the routine therapy of the diseases considered in this chapter; but already it has established itself as a potent means of combating streptococcal septicæmia.

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CHAPTER 67

PYOGENIC AND WOUND INFECTIONS

We have dealt in other chapters with many diseases which are associated with acute inflammatory or suppurative conditions in various parts of the body. There remain a number of infections which are characterized by the local formation of pus, by a spreading inflammatory reaction, associated with the formation of a purulent exudate, or by a septicæmic or pyæmic invasion of the blood stream. The bacteriology of some of the more important of these conditions is briefly discussed in the present chapter. It should be emphasized that the separation of bacterial infections, or of the bacteria which cause them, into pyogenic and non-pyogenic groups is largely arbitrary, and depends on the observation of the relative frequency of frankly suppurative lesions, among all those reactions which together characterize the association between a particular bacterial parasite and a particular animal host, the host species usually being man. It is quite easy to select some bacterial species, such as *Staph. aureus* or *Str. pyogenes*, as frankly pyogenic, and some others, such as *Myco. lepræ*, *C. diphtheriæ*, or *Cl. tetani* as non-pyogenic, in the usual sense; but there are very many pathogenic bacteria which cannot be assigned definitely to one class or the other. The reaction of acute inflammation, associated with the formation of a serous, or sero-fibrinous exudate, and with the diapedesis and accumulation of polymorphonuclear cells, is a fundamental defence mechanism, which is brought into play in response to a great variety of stimuli; and there are few bacterial species which are not pyogenic, in the sense that their presence may call forth this particular reaction in certain situations, and in certain circumstances. The characteristic reaction to the tubercle bacillus, for instance, takes the form of a non-suppurative granuloma, with giant cell formation and caseation; but in the pleural cavity, in the meninges, or in the urinary bladder, the same bacillus may cause the formation of an inflammatory exudate containing a considerable proportion of polymorphonuclear cells. Similarly, the typhoid bacillus is not, in the usual sense, a pyogenic organism; but it may cause a suppurative cholecystitis. All we can do, in discussing pyogenic infections in general, is to indicate in a few instances the bacteria which are most commonly associated with these conditions.

Septicæmia.

Bacteræmia, in the sense of the mere presence of bacteria in the blood stream, has been described in several earlier chapters. Septicæmia is here used as a designation for a clinical syndrome associated with a severe bacteræmic infection. We may note that, among those organisms which tend to produce pyogenic lesions terminating in a fatal septicæmia, *Str. pyogenes* is by far the most important. The pneumococcus, belonging to the same genus, is a frequent cause of the same type of infection, either

invading the blood stream directly from the lung, or from some suppurative focus such as an empyema. *Staph. aureus* may produce a purely septicæmic infection, following a localized infection, or an acute lymphangitis, but the generalization of a staphylococcal infection more commonly takes the form which is described in the following section. The occurrence of a transitory invasion of the blood stream by streptococci of the viridans type in cases of oral sepsis will be referred to in Chapter 68 (p. 1515). Attention may be drawn to the occurrence of septicæmia due to the non-sporing anaerobe *Fusiformus fusiformis*. This type is particularly liable to follow anginal infections, though it sometimes occurs after appendicitis or endometritis (see Chapter 79). Septicæmia due to anaerobic streptococci also occurs. Infections by anærobic bacteria are liable to be overlooked because diagnostic culture of the blood is seldom made anaerobically; and in the same way infections with bacteria that need an excess of carbon dioxide for growth (Chapter 3) will not be discovered unless a sample of blood is cultivated in air containing 5–10 per cent. CO_2 (see Khairat 1940).

The great majority of bacteria which produce suppurative lesions may on occasion give rise to a secondary septicæmia; and in almost all fatal cases of this type such an invasion of the blood stream occurs during the terminal stages of the disease. Thus a septicæmia occurs during the later stages of meningococcal meningitis, in cases of acute peritonitis, in rare cases of acute gonococcal infection, and so on. It is sometimes asserted that in septicæmia organisms are multiplying in the blood, while in bacteræmia they are derived from infected tissues. Such a distinction clearly cannot be drawn from the results of blood culture; it rests indeed on dubious clinical inference. The observation that a large and rapid increase in the number of circulating bacteria sometimes takes place just before death suggests multiplication in the blood stream; but with this exception, the septicæmic phase of any bacterial infection is probably the expression of a rapid and continuous invasion of the blood stream from the tissues, either from a single focus which is active and extensive, or from the multiple foci which have been established earlier in the disease. In the frankly suppurative diseases, in which the primary or secondary foci are accessible to surgical interference, the results of adequate drainage testify to the efficiency of the clearing mechanism of the blood stream, if continuous re-invasion can be prevented.

Pyæmia.

This term is employed clinically to designate a type of generalization the mechanism of which differs, to some extent, from that referred to above. In some bacterial infections, and particularly in the more severe forms of infection with *Staph. aureus*, such as acute osteomyelitis, metastatic abscesses may occur in such situations as the kidney, the brain, or the myocardium. In rare cases of appendicitis, or of other suppurative lesions in the abdominal cavity, multiple abscesses may occur in the liver, in direct relation to the portal vein—a condition known as "portal pyæmia," and usually due to infection with *Bact. coli*. In either case the dissemination appears to depend on the occurrence of a suppurative phlebitis at the site of the primary lesion. This extends directly as a progressive thrombosis; and particles of the infected thrombus, becoming detached, are carried by the blood stream until they reach some situation in which the anatomy of the blood vessels determines their impaction, where they give rise to secondary abscesses.

Staphylococcal Infections of the Skin and Subcutaneous Tissues.

Many of the localized pustular lesions of the skin are caused by staphylococci. The milder forms, such as acne pustules, are usually associated with a white staphylococcus (see Chapter 25). The more severe forms, such as boils or carbuncles, are almost always due to *Staph. aureus*. It may be noted that, in the acne pustule, the white staphylococcus is usually accompanied by the acne bacillus, which Fleming (1909) regards as the primary ætiological agent of the disease. For the isolation of the acne bacillus in the presence of staphylococci penicillin is said to be of value (Craddock 1942).

Infection of the sebaceous follicles in acne, and of the hair-follicles in superficial boils, appears to take place directly from the skin, without trauma. A more serious type of staphylococcal infection sometimes follows an insignificant traumatic infection with a virulent strain of *Staph. aureus*, taking the form of a rapidly spreading lymphangitis, with involvement of the regional lymph glands, and often terminating as an acute septicæmia. *Staph. aureus* may also give rise to suppurative mastitis in nursing mothers and to a form of pemphigus neonatorum in infants; both diseases may at times assume epidemic proportions in maternity homes (see Editorial 1936, Elliott, Gillespie and Holland 1941, Henderson 1943).

Botriomycosis.—In the past the term botriomycosis has been limited mainly to a disease of horses characterized by the occurrence of chronic abscesses in the skin, subcutaneous tissue, udder, spermatic cord, and less often the viscera. Of recent years, however, a disease of cattle and swine referred to as actinomycosis of the udder, has attracted considerable attention (see Magnusson 1928, Albiston 1936). Bacteriological study has rendered it clear that both the disease in the horse and the udder disease in cattle and swine are caused by *Staph. aureus*. There is therefore no longer any justification for calling two ætiologically identical diseases by different names, particularly since the term actinomycosis is applied to a disease of entirely different origin. We propose to use the term botriomycosis to cover all chronic granulomatous infections in animals due to *Staph. aureus*. A word of caution, however, is necessary. In swine, though not in cattle, true actinomycosis of the udder, due to infection with *Actinomyces bovis* or a closely related organism, does occur, and the differential diagnosis between this condition and botriomycosis has to be made by pathological and bacteriological methods. Again, *Staph. aureus* not infrequently gives rise in cows to an acute mastitis; this disease must not be referred to as botriomycosis.

In the horse the abscesses are single or multiple, have thick fibrous walls, and are liable to break down with the production of sinuses and fistulæ containing a gelatinous discharge. On section, the interior of the abscess is seen to consist of soft nodes of granulation tissue situated in a network of fibrous strands. Embedded in the granulation tissue are small yellowish-white granules about the size of a grain of sand. These consist of grape or mulberry-like clusters of Gram-positive micrococci surrounded by a zoogloeal substance. The granules are sometimes referred to as Bollinger's granules, and the organism has been called *M. ascoformans*, though there is little doubt that it is in fact *Staph. aureus*. A similar picture is seen in mammary botriomycosis of swine. In cattle, however, the colonies are surrounded by definite clubs instead of by an undifferentiated mass, and thus bear a close resemblance to true actinomycosis. In all three animals the disease may be reproduced by experimental inoculation of *Staph. aureus* under suitable conditions.

Streptococcal Infections of the Skin and Subcutaneous Tissues.

Erysipelas, an acute spreading infection of the true dermis, has been considered in Chapters 53 and 66.

Impetigo contagiosa, a contagious and sometimes epidemic infection of the superficial layers of the skin characterized by early vesicle formation and subsequently by weeping scabbed lesions, is usually thought to be streptococcal in origin. The evidence, however, in favour of this view is not altogether convincing. Cruickshank (1941) examined 23 cases, and isolated Group A haemolytic streptococci from 15 and *Staph. aureus* from 18 of them. Bigger and Hodgson (1943), who made 230 cultures from 130 cases, found that 97 per cent. of them yielded staphylococci and 32 per cent. *Str. pyogenes*. Staphylococci were present without streptococci in 67 per cent. of cultures, and *Str. pyogenes* without staphylococci in only 2 per cent. The longer the disease lasted, the higher was the proportion yielding streptococci, suggesting that these organisms were secondary invaders. In a later series of 50 cases, *Staph. aureus* was isolated from every patient, and *Str. pyogenes* from only 7 (Bigger and Hodgson 1944). These authors conclude that impetigo is primarily a staphylococcal infection.

Cellulitis is an acute spreading infection of the subcutaneous tissue, characterized by the formation of a sero-purulent, often blood-stained exudate, with no evidence of localization. Rapid lymphatic spread, and septicæmic generalization, are far more frequent than in the case of the localized staphylococcal infections. In very acute infections, such as those which may follow post-mortem wounds, the local manifestations may be minimal, the spread being so rapid that an acutely fatal septicæmia dominates the clinical picture from the first. In these conditions the streptococcus concerned is almost always *Str. pyogenes*.

It may be noted that a comparison of the modal types of staphylococcal and streptococcal infection affords an excellent example of the importance of differences in the biological characters of bacteria, in determining the type of lesion which they produce when they gain access to the tissues.

Mastitis in Cattle.—This important disease can only be touched on briefly here. Those seeking further information are referred to the monograph by Steck (1930) and to the series of papers by Minett and his colleagues in this country (Minett, Stableforth, and Edwards 1929, 1930, 1932, 1933, Minett and Stableforth 1931, Minett 1932, 1935, 1936, 1937, Stableforth 1930, 1932, Edwards 1932, 1933, 1934, 1938, Stableforth, Edwards, and Minett 1935, Report 1941, Watts, 1941, Discussion, 1942), to Klimmer and Haupt (1930) and Seelemann (1932) in Germany, to Rosell (1933) in Canada, to Hucker, Reed and Savage (1937) in the United States, and to Stewart (1937) and Bull, Murnane, Munch-Petersen and MacLean (1940) in Australia.

The disease is very prevalent among milch cows both in Europe and in the United States (see Brigham *et al.* 1929, Hucker *et al.* 1932), and constitutes one of the most serious economic problems confronting the dairy farmer. It is moreover of some public health importance, since occasionally streptococci of a type pathogenic for human beings may be excreted in the milk and give rise to scarlet fever or septic sore throat in those consuming it in the raw condition (see Chapter 93).

The bacteriology of mastitis is varied. Minett, Stableforth, and Edwards (1929), who investigated 113 cases of mastitis, found that 82 were due to streptococci, 21 to *C. pyogenes*, 6 to staphylococci, 3 to coliform bacilli, and 1 to *F. necrophorus*.

Ps. pyocyanea is sometimes responsible (Swett *et al.* 1944). We may briefly consider these different types.

STREPTOCOCCAL MASTITIS.—The streptococci causing mastitis may be divided on cultural, biochemical, and serological grounds into three groups.

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Group II streptococci give rise usually to acute or subacute mastitis, which is much less common than the chronic form just described. The distinguishing features of Group II streptococci are absence of hæmolysis, reduction of methylene blue in milk, failure to ferment salicin, mannitol, inulin, or æsculin, and poor production of acid in glucose broth. These organisms belong to Lancefield's Group C and are often referred to as *Str. dysgalactiæ* (Stableforth 1942). Contagion from the hands of the milker plays no obvious part in this type of mastitis. Usually only one quarter of the udder is affected. The condition is, however, often very acute, and may lead to the partial or complete destruction of the quarter involved.

We may also include in this group the sorbitol-positive trehalose-negative sub-group of Lancefield's Group C strains of hæmolytic streptococci (see p. 581).

Group III streptococci are a much less important cause of disease. They are characterized by absence of hæmolysis, production of acid in litmus milk at 10° C., reduction of methylene blue in milk, and fermentation of salicin, mannitol, and inulin. They do not fall into any of Lancefield's groups. They are sometimes referred to as *Str. uberis*.

Streptococcus pyogenes.—This organism is occasionally isolated from cows suffering from mastitis. The udder is probably infected from the nasopharynx of the milker or other human carrier. Its presence in the milk is likely to be followed by outbreaks of sore throat or scarlet fever in persons consuming it in the raw state (see p. 580 and Chapter 93).

MASTITIS DUE TO C. PYOGENES.—This organism is responsible for so-called "summer mastitis." The disease is rather more frequent during the second half of the year. It is far commoner in dry cows than in cows in milk, and usually develops shortly before calving. One or more quarters of the udder are affected. Constitutional symptoms, sometimes accompanied by swelling of the hock joints, are not uncommon. The condition is often chronic, and abscesses in the udder tissue may break through the skin. Microscopical examination of the udder secretion reveals enormous numbers of Gram-positive diphtheroid bacilli, which, when cultivated on coagulated blood serum, give the typical pitted appearance due to liquefaction.

STAPHYLOCOCCAL MASTITIS.—This form usually occurs a few days after calving. One or more quarters of the udder are affected. The disease is sometimes acute, accompanied by gangrene and may prove fatal. More often, however, it is of the chronic catarrhal type (see Minett 1937). The discharge from the affected quarters contains large numbers of hæmolytic staphylococci. Occasionally a chronic granulomatous condition develops; this is usually referred to as actinomycosis but is more properly termed botriomycosis.

Diagnosis of Mastitis.—In its more severe forms mastitis can be detected by clinical means alone, but the chronic streptococcal types of infection usually call for laboratory assistance. Individual quarter samples of milk are preferred for examination, the fore-milk being the most suitable. In well-marked cases of the streptococcal disease flakes in the fore-milk are visible against a dark background; the reaction of the milk is more alkaline than normal; on centrifugation of the milk the cellular sediment is found to be increased and to exceed 1 part per 1,000 by the Breed Smear method the cells usually number more than 5 million per ml.; microscopical examination of the centrifugal deposit reveals the presence of fairly long-chained streptococci; and cultivation of the centrifuged deposit shows the presence of streptococci which, on further examination, are found to belong to one of the mastitis groups. The most delicate test consists in the cultivation of the gravity cream or the centrifuged deposit, preferably in deep blood agar containing aesculin and crystal violet (Edwards 1933). For herd tests, composite milk samples from the udders of each cow may be incubated in the presence of sodium azide and bromcresol purple as a preliminary means of detecting milks containing *Str. agalactiæ* (Edwards 1938).

Prevention and Treatment of Mastitis.—The early observations of Minett and his colleagues led these workers to suggest a modified eradication plan for dealing with chronic streptococcal mastitis. The milk of every cow in the herd is examined culturally, and the affected animals are either sold out, segregated on another part of the farm, or, if neither of these courses is practicable, milked last. Strict attention must, of course, be paid to the general hygiene of milking, and every precaution taken to prevent infection of the clean herd. The rationale of this procedure is based on the assumption that *Str. agalactiæ* is an obligate parasite of the cow's udder. More recent work has thrown doubt on such a belief: the organism has, in fact, been found to survive for several weeks, and even months, outside the animal body (see Watts 1941), so that complete success by the eradication method could hardly be expected. Experience has shown this deduction to be true (see Hucker and Harrison 1937, Bull *et al.* 1940, Watts 1941). Nevertheless it is possible by these means to reduce greatly the incidence of overt disease.

Attempts to control infection by vaccine prophylaxis have not been promising (see Hucker and Hansen 1937). On the other hand, treatment of the affected cows by injections into the udder of bactericidal agents has proved very much more satisfactory. Acriflavine and gramicidin (see Watts 1941, Little *et al.* 1941) have both been used with success; but the best results appear to be obtained with sulphonamides; if these fail, then penicillin can usually be relied upon to effect a cure. Irrigation of the udder, which is often effective in streptococcal and staphylococcal infections (see Swett *et al.* 1944), is, however, time-consuming and entails the loss of several days' milk. For this reason there is an advantage in giving sulphonamide by the mouth. The oral administration of sulphanilamide may be recommended for cows infected with *Str. agalactiæ*, though not all cases respond favourably to this form of treatment (Stableforth *et al.* 1942).

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It is convenient to discuss in this section a few examples of streptococcal infection of animals, some of them contagious.

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Streptococcal mastitis is sometimes responsible for severe outbreaks. We may briefly consider the different types.

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Group II streptococci give rise usually to acute or subacute mastitis, which is more rapid in onset than the chronic form just described. The distinguishing characteristics of Group II streptococci are absence of haemolysis, reduction of methylene blue, failure to ferment salicin, mannitol, inulin, or æsculin, and poor production of acid in glucose broth. These organisms belong to Lancefield's Group C and are sometimes referred to as *Str. dysgalactiae* (Stableforth 1942). Contagion from milker to milker plays no obvious part in this type of mastitis. Usually only one quarter of the udder is affected. The condition is, however, often very acute and may lead to the partial or complete destruction of the quarter involved.

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lymph nodes are mainly affected; they are converted into large, thick-walled, fluctuating abscesses containing grumous, pale yellow, granular pus. The disease is generally chronic, and may be confused with pseudotuberculosis (see Chapter 73). Rae (1936) reports a spontaneous epizootic in guinea-pigs, spreading by contact, also due to a β-hæmolytic capsulated streptococcus. It is characterized by great enlargement of the cervical lymph nodes of a non-suppurative type, and multiple necrotic yellowish areas in the myocardium.

In dogs, infection with Group G streptococci may manifest itself as a febrile adenitis of the submaxillary or cervical lymph nodes, which sometimes suppurate, or by an infection of the genital tract in bitches, resulting in sterility or abortion. The infection may be complicated by a fatal septicæmia (Hare and Fry 1938).

A type of alpha-hæmolytic streptococcus, serologically related to Lancefield's Group C streptococci, was found by Blakemore, Elliott and Hart-Mercer (1941) in suppurative polyarthritic lesions of lambs suffering from *joint-ill*. In some animals the polyarthritis was accompanied by an infective endocarditis. Normal lambs were susceptible, but convalescent lambs and adult sheep were immune to experimental infection with the streptococcus.

Group C streptococci are commonly found in infections of the horse. Bazeley and Battle (1940) described five sub-types of equine Group C streptococci, all of which were associated with respiratory catarrhs, wound infections and suppurative lesions in various parts of the body. Their Type 1 appeared to be the causal organism of *strangles*, a suppurative lesion of the upper respiratory tract, characterized by abscesses in the throat and submaxillary region. Bazeley (1940) was able to immunize mice against infections by this organism, using vaccines of heat-killed capsulated streptococci.

Conjunctivitis.—One of the commonest causes of acute conjunctivitis is the Koch-Weeks bacillus, which seems to be identical with *H. influenzæ* (see Chapter 33). This condition appears to be widespread, occurs in epidemic form, and shows a definite seasonal prevalence in different countries. It is particularly common in Egypt, where the bacillus was first isolated by Koch. Another, less common, cause of conjunctivitis is the pneumococcus. This type of infection, which is more severe than that due to the Koch-Weeks bacillus, is irregular in distribution, is confined chiefly to children, and may occur in localized epidemics. It is more common in Eastern than in Western countries. Subacute, or angular, conjunctivitis is due to the Morax-Axenfeld bacillus (see Chapter 34). Gonococcal conjunctivitis—ophthalmia neonatorum—is described in Chapter 65. (For a general account of the bacteriology of conjunctivitis see Axenfeld 1928.)

Suppurative Lesions in Bones and Joints.—Acute osteomyelitis, occurring independently of a compound fracture, is almost always due to infection with *Staph. aureus*. It occurs mainly in children, in whom it not infrequently develops after a simple blow, or similar trauma. The infection is blood-borne. Acute suppurative periostitis is rare. When it occurs, it is usually a streptococcal infection. A subacute, or chronic, periostitis occasionally occurs as a sequel of typhoid, or paratyphoid fever, and is caused by the bacillus responsible for the primary infection. Such abscesses occur most commonly over the ribs, or the cranium.

A metastatic purulent arthritis may occur as a sequel to infection with any pyogenic bacterium; though the pneumococcus is the organism most frequently isolated in such cases. Other bacteria are rarely isolated from true suppurative arthritis in man, except in those cases which follow wounds entering the joint. In animals other bacteria are often responsible for this condition, as, for instance, *Corynebacterium pyogenes* and *Erysipelothrix rhusiopathiæ*.

Suppurative Lesions of the Alimentary Tract.

Appendicitis.—The bacteriology of acute appendicitis, and of the associated appendix abscesses, is very confused. It is possible that the primary lesion occurs in the wall of the appendix, and that it is due to a blood-borne infection with a streptococcus of the viridans type (see Rosenow 1915). The complex bacterial flora of this part of the intestine ensures a rapid secondary invasion of the damaged tissue with a host of different bacteria, among which *Bact. coli* is prominent; and the bacteriology of the lesion found at operation is correspondingly complex. Veillon and Zuber (1898), using a combination of aerobic and anaerobic methods, examined 22 cases of appendicitis. From 19 cases they isolated anaerobic bacilli associated with rare streptococci and coliform bacilli; from 2 of the cases anaerobic bacilli alone were cultured. Usually 5 or 6 different species were found together, and never less than two. Of the anaerobic bacilli the majority belonged to the Gram-positive non-sporing group, described in Chapter 18; amongst these *Fusiformis fragilis*, *F. ramosus*, *F. fusiformis*, and *F. furcosus* were the commonest. *Cl. welchii* was also found very frequently. More recently Weinberg and his colleagues (1926, 1928) examined 150 cases of acute appendicitis, a great number of which were of the gangrenous type. Of the aerobic bacteria the frequency was *Bact. coli* 128, enterococcus 41, *Proteus* 14, *Staphylococcus* 12, and *Streptococcus* 10; a few other species of organisms were found occasionally. Of the anaerobic bacteria the frequency was *Cl. welchii* 49, Gram-negative rod-shaped organisms 60, cocci and streptococci 40, *F. ramosus* 16, *Cl. fallax* 5, *Cl. septicum* 2, *Cl. histolyticum* 2, and other organisms in smaller numbers. Essentially similar results have been recorded by Schmitz (1930) and Buttiaux and Tiprez (1933). It is clearly impossible on the present evidence to assess the rôle played by each of these organisms in the ætiology of appendicitis, but it would appear not improbable that the organisms responsible for the gangrene and the general toxæmia in severe cases belong mainly to the anaerobic groups. Weinberg and his co-workers (1928) are so impressed with the importance of the anaerobes that they have recommended the use of antitoxic serum prepared against these organisms in the treatment of appendicitis (see Chapter 78).

Cholecystitis.—The frankly suppurative form of cholecystitis appears to be due, in the majority of cases, to infection with *Bact. coli*, sometimes associated with streptococci. Acute cholecystitis may follow infection with *Bact. coli*, with *Salm. typhi*, or with bacilli of the paratyphoid group. The relation between infection of the gall-bladder and the carrier-state in typhoid fever, will be discussed in Chapter 69. It may be noted that infections of the gall-bladder provide an interesting example of the importance of local conditions, of a semi-mechanical nature, in originating or perpetuating bacterial infection. Bacteria, and particularly members of the coli-typhoid group, have frequently been isolated from the interior of gall-stones. It is highly probable that a mild bacterial infection so alters the local conditions in the gall-bladder as to favour the formation of gall-stones, and that the cholesterol and bile salts of which these are composed may be deposited on a central nidus of desquamated cells, or fibrin, containing bacteria. There are also good reasons for believing that the presence of gall-stones greatly increases the liability to bacterial infection; so that we have a vicious circle, each abnormality tending to promote, or perpetuate, the other. The bacteriology of gall-bladder infections is also of interest, in that it provides an example of the selective action of certain body fluids on bacteria, and illus-

trates the necessity for making due allowance for such factors in interpreting bacteriological findings. We have noted elsewhere that some authorities believe that the typhoid bacillus gains access to the gall-bladder *via* the blood vessels of the wall of the viscus, and not by the bile ducts. It has been suggested by many observers that the same is true in the majority of cases of cholecystitis, and that the primary lesion is a focus of infection beneath the epithelial lining. Rosenow (1916) has suggested that streptococci, with a special tendency to localize in the gall-bladder wall, play an important part in the production of cholecystitis in man. Wilkie (1928) has recorded an interesting series of observations, which tend to support this view. In 50 cases of cholecystitis, mainly chronic, he took cultures from the bile, the gall-bladder wall including all coats, the submucosa, and the small lymphatic gland situated near the junction of the cystic duct and the gall-bladder. From the bile, and from the pieces of gall-bladder wall which were contaminated with bile, he isolated streptococci on two occasions, *Bact. coli* three times, and *Cl. welchii* once. From the submucosal cultures, in which special care had been taken to prevent contamination with bile, he isolated streptococci in 21 cases, and *Cl. welchii* once. From the cystic gland he isolated streptococci in 43 cases, *Bact. coli* once, and *Cl. welchii* once. He concludes that cultures taken from the bile give an entirely misleading picture of the essential bacteriology of cholecystitis, owing to the inhibitory action of the bile salts on the streptococcus, which is the cause of the primary infection. These observations clearly suggest that *Bact. coli* may, in many cases, play the part of an important secondary invader, favoured by the selective action of the bile, rather than that of a primary agent.

Peritonitis.—Some cases of peritonitis are primary, for example, certain cases in children due to infection with the pneumococcus; but the great majority of cases are secondary to some primary intra-abdominal lesion, such as appendicitis, perforated gastric or duodenal ulcer, or some type of intestinal obstruction. The species of bacteria concerned in the primary invasion of the peritoneum depends in part on the nature of the primary lesion, in part on the flora of the section of the intestinal tract involved. In intestinal obstruction the bacteria from within the intestine tend to make their way through the intestinal wall, and to produce a mixed infection. In such cases, and in the later stages of any infection of the general peritoneal sac, other than those which are acutely fatal, the bacteriological picture is usually dominated by the colon bacillus.

Fatal post-operative peritonitis is often due to infection with a hæmolytic streptococcus, and a similar infection may occur as part of a septicæmic infection. Pelvic peritonitis, following salpingitis, is usually due to the gonococcus. Other bacteria which may be found in the peritoneal exudate include *Staph. albus*, bacilli of the *Proteus* group, and *Ps. pyocyanea* (see Dudgeon and Sargent 1905). In those cases in which free transit has occurred from the interior of some part of the lower intestine, anaerobes of the *Fusiformis* and *Clostridium* groups may be present. Cazzamali and Miglierina (1933), who examined 81 cases of acute peritonitis, found that aerobic organisms accounted for about two-thirds and anaerobic for about one-third of the total number isolated. In point of time the aerobic organisms preceded the anaerobic. *Bact. coli* seemed to be able to diffuse more rapidly through the peritoneal cavity than any other organism, while streptococci persisted for the longest time. In cases of perforated gastric or duodenal ulcer no organisms could be detected for 6, or even 12 hours, but after this time the peritoneal

cavity was always infected, the commonest organism being a non-hæmolytic streptococcus. From a prognostic point of view, excluding infections with *Str. pyogenes*, the fewer organisms and the fewer species there are present, the more favourable is the outlook (see also Meleney *et al.* 1931).

Suppurative Lesions of the Respiratory Tract.

Suppurative lesions of the accessory sinuses of the nose usually arise by direct extension from the nares or nasopharynx, and the bacteria most frequently responsible are the potentially pathogenic constituents of the nasopharyngeal flora—*Str. pneumoniæ*, *Str. pyogenes*, and *H. influenzæ*. Another organism frequently isolated from cases of suppurative sinusitis is *Staph. aureus*; while acute infection of the nasal sinuses with the meningococcus has been described, and is believed by some observers to play a part in the genesis of cerebrospinal meningitis (see Chapter 64).

Under this heading, we may, for convenience, include otitis media. The great majority of cases in man are caused by *Str. pyogenes*, *Str. pneumoniæ*, or *Staph. aureus* (Fisher 1929); occasionally enterococci are responsible (Rantz and Kirby 1943). Middle-ear disease is very common in rats, particularly in adult animals; the main organisms involved appear to be *Actinobacillus actinoides*, streptococci, and diphtheroids (Nelson and Gowen 1930).

The bacteriology of infections of the lungs is discussed in Chapter 74. Suppurative inflammation of the pleural cavity arises by direct extension from the pulmonary lesion, and is due to the same infecting agent—in most cases of lobar pneumonia to *Str. pneumoniæ*, of secondary broncho-pneumonia to *Str. pneumoniæ*, *Str. pyogenes*, or occasionally to some other organism. The bacterial flora of chronic bronchiectatic cavities in the lung is mixed, pyogenic organisms and throat flora usually predominating. Allison, Gordon and Zinnemann (1943) have produced evidence suggesting that, among respiratory pathogenic bacteria, *H. influenzæ* is most commonly associated with these lesions.

Pneumonia is common in adult rats and appears to be due to very much the same organisms as those responsible for otitis media. A pneumococcal pneumonia occurs in guinea-pigs debilitated by pregnancy or an ill-balanced diet; the infection, which is usually due to Type XIX pneumococci, may also take the form of a respiratory catarrh, or give rise to a uterine lesion (see Petrie 1933). It may be noted that Jowett (1930, 1931) has described a *chronic pyobacillosis of sheep*, met with in the abattoir, which is characterized by multiple small abscesses in the lungs and sometimes other parts of the body. The causative organism is a Gram-negative ovoid bacillus, said to be identical with the *Bact. purifaciens* of Christiansen.

Acute Suppurative Lesions of the Urinary Tract.

Acute suppurative infections of the urinary tract leading to pyelitis, pyelonephritis, and cystitis are caused most frequently by *Bact. coli*. Dudgeon, Wordley, and Bawtree (1921, 1922–23) have drawn attention to the frequency with which hæmolytic types of *Bact. coli* are found in such infections, and have noted that these hæmolytic strains are particularly frequent in males. In a series of 44 *coli* infections in males, 72·7 per cent. were due to the hæmolytic and 27·3 per cent. to the non-hæmolytic type; in 116 female cases, on the other hand, only 31 per cent. were due to the hæmolytic, and 69 per cent. to the non-hæmolytic type. More recently Dudgeon and Pulvertaft (1927) have described a particular type

of acute urinary infection, associated as a rule with acute pyelitis or pyelonephritis, and caused by a hæmolytic, slow-lactose-fermenting type of coliform bacillus. The general symptoms at the onset are so severe that the urinary affection is often masked, and the true nature of the disease is not manifest for 2 or 3 days, when pus is found in the urine. In most cases recovery occurs, and the urine clears up completely, contrasting in this respect with infections due to the typical non-hæmolytic *Bact. coli*, in which chronic bacilluria is the rule.

Bact. aerogenes is said to be frequently present in infections of the genito-urinary tract (Hill *et al.* 1929, Wernig 1939 ; see also p. 2027). Another common invader is *Proteus vulgaris* (Taylor 1928) ; in acute cases this organism can be isolated from the blood stream, and may be present in pure culture in the urine. *Proteus vulgaris*, fæcal streptococci, *Ps. pyocyanea* and staphylococci are not infrequent secondary invaders in infections of the urinary tract. Infection of the urine with the gas-producing *Cl. welchii* may occur in cases of carcinoma of the rectum or bladder, associated with a recto-vesical fistula. Cystitis is a not uncommon complication of gonorrhœa, and is then due to the gonococcus, alone or in association with other organisms. Tuberculous cystitis or pyelonephritis, which is itself associated with pyuria, is frequently complicated during its later stages by a secondary infection with *Bact. coli*, staphylococci, or other organisms.

Infections of the bladder and of the pelvis of the kidney provide another illustration of the importance of local mechanical factors in promoting bacterial infection. Renal or vesical calculi, enlarged prostate, urethral stricture, vesical papilloma or carcinoma, are all conditions which, sooner or later, are associated with an infective cystitis. *Staph. aureus* is the organism most commonly found in urinary calculi, both in the stone itself, and in the adjacent tissues (Hellström 1924, Winsbury-White 1935).

Pyelonephritis is sometimes observed in *cattle* and is characterized by a membranous necrotic inflammation, which attacks principally the papillæ and leads to progressive destruction of the kidney ; there is an accompanying inflammation of the capsule, and often considerable fibrosis of the pelvis of the kidney, the ureter, and the bladder. The ætiology of this condition is still uncertain. By some it is said to be due to a Gram-positive diphtheroid bacillus, *C. pyogenes* ; by others to a mixed infection with *C. pyogenes*, streptococci, coliform bacilli, and other organisms (Glage 1928, Jones and Little 1930).

Infection of Wounds

Bacterial infection is a common sequel to wounding. All wounds, from trivial cuts to the extensive areas of destruction that follow burning, are liable to infection, though in general the larger and deeper the wound and the more damaged and " devitalized " the wounded tissues, the greater is the susceptibility to infection. The risk of infection to a clean operation wound, closed so as to heal by first intention, is relatively small ; and that of an extensive compound fracture, with crushing or laceration of the tissues, is high. Few organisms are introduced into a clean operation wound, and after operation the wound can be adequately protected against subsequent infection. The same is true of the " physiological " wounds in the genital tract of the post-parturient woman ; sepsis does not develop if precautions are taken against the introduction of bacteria during and after labour. The accidental wound, whether inflicted in civil life or in war, differs in that the tissues are usually more damaged, and in the greater likelihood that the wound

will be contaminated by bacteria present in the environment of the wound at the time of infliction. The bacteria include those from the skin and clothes of the wounded person, and those from the soil, dust, dirt and other debris that fall on or are driven by force into the wound. Infection in these wounds is much commoner and has been studied more extensively than has infection of clean wounds. There is, however, no essential distinction between the two kinds, and our conclusions will apply with varying force to infection of all open lesions of the body.

It will be convenient to make an arbitrary distinction between the bacteria we find in a wound within a few hours of infliction, and those we find later. In the fresh wound, bacteria have had little time to multiply and elaborate their toxins, and there is practically no evidence of an inflammatory tissue response. Hence, with few exceptions we can regard the bacteria as contaminants. After a few hours, however, if signs of inflammation or other tissue response appear, we must consider them no longer as contaminating but as infecting bacteria. With this distinction in mind, it remains to determine the relation of the contaminating to the infecting organisms.

Wound Contamination.—If we except wounds grossly soiled with materials which, like road-dust, earth, saliva or fæces, contain large numbers of bacteria, the freshly inflicted wound contains relatively few viable organisms. But, though the numbers are small, a variety of different species is usually present; and few wounds are completely sterile. The frequency with which different bacteria are isolated depends partly on the methods of cultivation employed, and partly on the environment of the wound at the moment of its infliction. In general, micrococci and staphylococci other than *Staph. aureus* are most commonly found. Other frequently isolated species are spore-bearing bacilli (both aerobic and anaerobic), enterococci, α-hæmolytic and non-hæmolytic streptococci, diphtheroids and Gram-negative intestinal bacilli. (See Pulaski, Meleney and Spaeth 1941, Spooner 1941*b*, Hare and Willits 1942, de Waal 1943, Altemeier and Gibbs 1944.) Of the more pathogenic bacteria, *Staph. aureus* is present in up to 20 per cent. of wounds, and *Str. pyogenes* (Lancefield's Group A) in only a small proportion (see Williams and Miles 1945). *Cl. welchii*, though relatively common, behaves in the great majority of wounds as a saprophyte, and dies out fairly rapidly (see Chapter 78). So, in fact, do most of the contaminating species mentioned above, including *Staph. aureus* and *Str. pyogenes*, especially if dead tissue and foreign matter are removed from the wound. The results of repeated cultivation of the flora at intervals after infliction of the wound suggest that only in a small proportion of surgically treated wounds do the initially contaminating pathogenic bacteria persist and give rise to infection.

Wound Infection.—The bacterial flora of the infected wound is both qualitatively and quantitatively different. The organism most commonly found is *Staph. aureus*, which is present in the majority of open infected wounds (see, for example, Levaditi *et al.* 1939, Miles *et al.* 1940, Pulvertaft 1943, Meleney 1943). Next in frequency come *Str. pyogenes*, *Bact. coli*, *Proteus vulgaris*, *Ps. pyocyanea* and enterococci; the precise frequency will vary with the type and age of the wounds studied (see Altemeier 1942). In the large war wounds, as Fleming (1915) showed, the primary stage of infection, which lasts about one week, is due mainly to bacteria of fæcal origin, including spore-bearing bacilli; in the second, intermediate stage, these organisms are gradually replaced by pyogenic cocci; and the third stage is characterized by a suppurating infection with streptococci and staphylococci. But there is no general rule that applies to all the observed varieties, both clinical and bacteriological, of wound infection. Heavy infections with coliform bacilli may occur, particularly in large wounds of the trunk, buttocks and thighs (de Waal 1943); whereas in wounds of the extremities coliform infections are rare (see Williams and Miles 1945). In addition to the common pyogenic organisms and the gas-gangrene or tetanus bacilli noted above, wounds may, for example, become infected with anaerobic

streptococci, diphtheria bacilli, actinomyces, organisms of the *Hæmophilus* group and so forth. Among the anaerobic streptococcal infections we may note a gangrenous myositis, resembling clostridial gas gangrene in many respects, which is apparently due to the combined invasion of wounded muscle by *Str. pyogenes* and anaerobic streptococci (MacLennan 1943*a*, *b*). Wound diphtheria is uncommon, but when it does occur, it may assume epidemic proportions in hospital wards (Bensted 1936).

It must be emphasized that wounds may become infected without any obvious clinical manifestation of disease except perhaps a prolongation of the healing time. Miles (1944), for example, records that 29·0 per cent. of 282 small industrial wounds were latently infected in this way, mainly with *Staph. aureus*. The existence of such wounds is clearly of epidemiological importance, being analogous to the healthy carrier of disease in other infective conditions.

The Sources of Pyogenic Wound Infection.—The three chief bacterial causes of wound sepsis are *Staph. aureus*, *Str. pyogenes* and Gram-negative intestinal bacilli. We have already considered the distribution of *Str. pyogenes* in Chapter 66. We may recall that it is present in the throat of 5–10 per cent. of healthy persons and in the nose of less than 1 per cent.; and that persons with open streptococcal lesions—scarlet fever, tonsillitis, and streptococcal skin diseases—contribute large numbers of cocci to their surroundings. Streptococcal wounds are also prolific sources of this organism. Under ordinary conditions, however, *Str. pyogenes* is found infrequently and in small numbers on the skin and clothes, even of carriers (see Hare 1941)—a fact in accord with the very low incidence of *Str. pyogenes* in freshly inflicted wounds. Coliform bacilli are present on the skin near the anal region, and it is reasonable to suppose that large trunk and buttock wounds are often infected by fæcal organisms on the neighbouring skin. In *Staph. aureus* we have a potentially pathogenic organism existing normally in close association with man, at least in the temperate regions where the carriage of this organism has been investigated. The distribution of *Staph. aureus* in man is considered in detail in Chapter 90. It is sufficient to note here that the observed carrier rates for adults are about 50 per cent. for the anterior nares, and 5–20 per cent. for the skin of the hands (see Gillespie *et al.* 1939, Miles *et al.* 1944). A smaller percentage of persons carry it on the skin in various other parts of the body (Martin 1942, R. E. O. Williams, personal communication). Skin carriers are usually nasal carriers; and, in view of the persistence and profuseness of nasal carriage, it would appear that the nose is the primary source of the external *Staph. aureus* of the body, and that the skin is secondarily contaminated from the nose.

Miles and his colleagues (1944) have observed that wound contamination by *Staph. aureus* is commoner among skin carriers than among those whose skins are free from the coccus; and, in the majority of cases tested, the skin staphylococcus was of the same bacteriophage type as the wound staphylococcus. The fact that nasal and skin strains in one person usually belong to the same serological or phage type (Gillespie *et al.* 1939, Fisk and Mordvin 1944, Miles *et al.* 1944) strongly suggests that both contamination and infection of wounds by the patient's own staphylococci are common.

The incidence of infection by these organisms in older wounds is far higher than we should expect from the initial contamination rates. Moreover, in the severer wounds, the infection rates with various organisms increase with the age of the wound. Thus, in the war of 1914–18, it was observed that hæmolytic streptococci were present in about 15 per cent. of pre-operative wounds, and in as many as 34·6 per cent. (Stokes and Tytler 1918–19) and 90·0 per cent. (Fleming and Porteous 1919) of wounds after a week or more of post-operative treatment. Similar observations with respect to *Str. pyogenes*, *Staph. aureus*, coliform bacilli, and other organisms, were made in the second world war (see Memorandum 1941, Spooner 1941*a*); and it appears that in a large number of cases, the infections are added after the infliction of the wound. For the most part the evidence of added infection is circumstantial, for there is no direct means of excluding the possibility that the late-appearing infections arise from bacteria that have lain dormant in the wound since its infliction, but which eluded bacteriological detection by reason of the small

number of organisms present. With *Str. pyogenes*, however, the evidence is more direct. As in epidemiological surveys of other streptococcal diseases (Chapter 66), the identification of the various Griffith types of *Str. pyogenes* has made it possible to prove that infection is often due to streptococci added in hospital. There is, then, good reason to believe that in a large number of instances where the precise infecting organism cannot be traced to its source, late-appearing infection is, in fact, due to added infection (see Miles 1944). Not only may a clean wound become infected, but one type of infection may be superimposed on, and eventually replace another. Thus the successive predominating organisms in a wound may, for example, be micrococci, *Staph. aureus*, *Str. pyogenes*, *Ps. pyocyanea* and *Proteus vulgaris*, and finally, immediately before healing, *Staph. albus* and diphtheroids.

The establishment of this point of view is important, for it clearly indicates that antibacterial measures at the time of wounding, the removal from the wound of foreign matter and of devitalized tissues, and the application of cleansing and antibacterial agents, are insufficient safeguards against wound infection. The wound must be protected against the risks of infection until it is fully healed.

The risks are greatest in casualty stations, hospitals and other institutions. They are relatively low in civilian surgical practice, for there are few reservoirs of infection, and most clean operation wounds offer too small a surface for the successful implantation of organisms; but even here cross-infection of the larger, chronic, open lesions like tuberculous sinuses, and abscess cavities after surgical interference, may occur. War and accident wounds are more susceptible to infection, and in war-time the large numbers of infected wounds in hospital wards ensure reservoirs of infection far larger than those of the normal nose, throat and skin carriers of pyogenic cocci. Consequently, added infection of wounds is numerically a greater problem in war. Such infections appear to be transmitted mainly by contact or droplets. *Str. pyogenes*, *Staph. aureus* and coliform bacteria are often found in the air of surgical wards, but their rate of sedimentation is slow and few are likely to fall on to a small wound during its exposure for dressing or inspection; consequently, air-borne infection is of minor importance compared with contact infection. Burns, and other extensive wounds that are exposed for long periods during treatment, appear to be far more susceptible to air-borne infection (Cruickshank 1935, Colebrook and Francis 1941). Precautionary measures comprise aseptic routines carefully devised to meet the known risks of added infection, including air-borne infection in appropriate cases (Memorandum 1941), and the local application of antiseptic or chemotherapeutic substances (see below).

Treatment of Pyogenic Infections

Surgical.—Although the discussion of surgical methods of treatment takes us beyond our proper ground, it is permissible to point out that general bacteriological principles indicate the paramount importance of judicious surgical interference in those acute suppurative infections which are accessible to such measures. The removal, or adequate drainage, of a localized focus of infection will usually enable the defence mechanisms of the body to deal satisfactorily with the remaining bacteria. We have emphasized above the importance of mechanical factors in many cases of acute or chronic pyogenic infections; and the rectification of these abnormalities by surgical means frequently results in a bacteriological cure. It may be noted that other factors, in addition to the removal of living bacteria, are involved in the successful drainage or removal of an infected focus. Reference to the discussion on "aggressins" in Chapter 48 will indicate that the antigenic constituents liberated in such a focus may play a significant part in extending the area of infection, or in favouring its local persistence.

Chemotherapy.—In recent years, a large variety of antibacterial agents has become available for treatment of pyogenic infections. Those with a high thera-

peutic index like the sulphonamides or penicillin may be used for both systemic and local treatment, while active but more toxic substances, like tyrothricin or the acridine compounds are fit only for local application—usually to open lesions (see Chapter 6).

It is beyond the scope of this chapter to discuss the chemotherapy of pyogenic infections in any detail. The sulphonamides have been extensively used, and have proved to be effective in the treatment of streptococcal infections. Staphylococcal infections also respond, particularly to compounds like sulphadiazine and sulphathiazole. Streptococcal septicæmia appears to be more readily influenced than staphylococcal septicæmia by the sulphonamides. These drugs are less effective against infections by Gram-negative bacilli, though their clinical effect is by no means negligible, especially in infections of the urinary tract (see Spink 1942). Penicillin is particularly effective in staphylococcal and streptococcal infections (see, for example, Christie *et al.* 1944, Dawson and Hobby 1944, Herrell 1944, Bloomfield *et al.* 1944).

Local therapy of abscesses, skin lesions, infected wounds, and so forth is practicable, and may be necessary when, for various reasons, it is impossible to maintain a blood concentration of the drug sufficient to ensure a therapeutic concentration in the infected organ. The efficacy of local sulphonamides upon susceptible bacteria depends in part upon their solubility, their activity at the pH of the infected lesion, and the extent to which they are antagonized by substances present in exudates and pus (see Chapter 6). Their toxicity for tissues is low. Penicillin preparations are almost entirely non-toxic, and are not conspicuously antagonized by exudates and pus. (For references see Memorandum 1944). Among other substances that appear to be of value in the treatment of open septic infections we may note tyrothricin (see Herrell and Heilman 1941, Rammelkamp 1942); propamidine (Thrower and Valentine 1943, Clark *et al.* 1943), and acridine compounds (Mitchell and Buttle 1942, Browning 1943).

It should be noted that in the chemotherapy of mixed infections, the relatively insusceptible organisms like *Bact. coli*, *Proteus vulgaris* and *Ps. pyocyanea*, which remain in the lesion after the suppression of the pyogenic cocci, may cause serious and sometimes fatal infection.

Jensen and others (1939) reported that the introduction of sulphonamide into compound fracture wounds protected against subsequent infection; and both local and systemic sulphonamides have been used prophylactically in wounds and in the peritoneal cavity at operation (see Spink 1942). It is difficult to assess their value in preventing the infection of war wounds, where there are so many factors, including concomitant methods of treatment, to take into account; but there is a general consensus of opinion that they are of value (but see Meleney 1943).

The induction of drug-fastness in bacteria subjected to less than bacteriostatic concentrations of antibacterial agents is a possibility to be borne in mind in the treatment of open lesions. There are on record a number of ward epidemics of streptococcal infection of burns and wounds, caused by a drug-fast strain of *Str. pyogenes* (see Colebrook *et al.* 1944); but, though there was in each case ample opportunity for the therapeutic induction of drug-fastness in the epidemic strain, no proof that it had become drug-fast under these conditions was in fact forthcoming.

Serotherapy.—The probability that staphylococci exert their deleterious action on the tissues by means of toxins holds out some hope of the value of serum treatment. There is evidence (Chapter 46) that resistance in the experimental animal to the bacterial infection is associated with a high antitoxin content of the blood;

this presumably acts by protecting the cells of the host against the necrotic effects of the α-toxin elaborated by the invading *Staph. aureus* (see Rigdon 1936). Antitoxic serum, capable of neutralizing the hæmolytic, skin-necrosing, lethal, and leucocidal properties of the toxin, can be prepared by inoculation of horses with formolized, and later with pure, toxin. Its α-antitoxin content can be titrated in one or other of the following ways.

(1) By testing mixtures of toxin and antitoxin for their hæmolytic activity towards rabbit red blood corpuscles. In practice the test dose of toxin (LH) is determined which, when mixed with one provisional unit of antitoxin, causes partial hæmolysis; this test dose is then used for titrating the unknown antitoxin; (2) by inoculating toxin-antitoxin mixtures in 0·2 ml. quantities intracutaneously into guinea-pigs of 300–400 grams. The test dose $\frac{Lr}{10}$ is the quantity of toxin which, when mixed with 0·1 provisional unit of antitoxin, causes a small but characteristic necrotic lesion in 24–48 hours; (3) by inoculating toxin-antitoxin mixtures in 0·5 ml. quantities intravenously into mice. The test dose employed is that quantity of toxin which, when mixed with one provisional unit of antitoxin, causes death of half the mice within three days; (4) by inoculating toxin-antitoxin mixtures in 0·5 ml. quantities intraperitoneally into mice. The procedure is similar to method (3). The international unit is now defined as the specific antitoxic activity contained in 0·5 mgm. of the dry standard preparation preserved at the National Institute for Medical Research, London. (See Hartley and Smith 1935, Smith 1938). Antisera also contain variable amounts of antibody to the leucocidin described by Panton and Valentine (1932), who ascribe the beneficial effects of staphylococcus antitoxin in part to its anti-leucocidin content (see also Valentine 1936). As mentioned on p. 616, this leucocidin is to be distinguished from the Neisser-Wechsberg leucocidin, which is reported to be similar to the α-toxin.

Though staphylococcal antitoxin has been generally available for some years, information about its value is scanty. It appears to be most effective in the treatment of severe local or generalized infections accompanied or likely to be followed by septicæmia, though only in a proportion of such cases is there a favourable response. The intravenous injection of large doses, in some instances totalling 600,000 units, is recommended, together with drainage of accessible lesions, whether in the soft tissues or bones (Dolman 1934*a*, *b*, Joyner and Smith 1936, Baker and Shands 1939). Goldberg and Bloomenthal (1941) report a synergic action of antitoxin and sulphonamides in the treatment of staphylococcal septicæmia.

Julianelle (1939, 1942) reports a beneficial effect in severe staphylococcal infections of an antiserum prepared against Type A staphylococci (see Chapter 25), most marked in cases of osteomyelitis accompanied by septicæmia.

Antiscarlatinal (antitoxic) and the ordinary type of antistreptococcal serum, which have already been described in relation to the treatment of scarlet fever and puerperal sepsis (Chapter 66), have on the whole given disappointing results in cases of septicæmia.

Vaccination.—Examination of the blood serum of man and animals has shown the presence in small amounts of natural antitoxin to staphylococci (see Bryce and Burnet 1932, Parish, O'Meara, and Clark 1934, Murray 1935, Nélis and Poncelet 1935, Gernez and Pannequin 1937, Weiss 1939). In normal human adults and new-born babies the antitoxin titre expressed in terms of international units is generally about 0·25–0·75 units per ml. In patients suffering from superficial staphylococcal infections, such as acne, blepharitis, furunculosis, and sycosis, the average titre is very slightly higher or practically unaltered. In cases of

carbuncles the titre is often slightly raised, while in cases of chronic osteomyelitis it is generally high, though not always so (see Blair and Hallman 1935–36), and may reach 15 units or more per ml. Experimentally and clinically it has been found that treatment of animals and patients suffering from chronic staphylococcal infections with formolized toxin—toxoid—but not with staphylococcal vaccines, is often followed by a rise in the antitoxin titre and considerable improvement in the local condition; though there seems to be no definite relationship between the absolute titre attained and the retrogression of the infection. The preparation of the toxoid requires care, and strict precautions must be taken to ensure its innocuity and its antigenic potency (Dolman and Kitching 1935, 1936, Smith 1936, 1937, Farrell 1941). The dosage recommended by different workers varies somewhat. Dolman (1933, 1935) starts with a dose of 0·05 ml., increases by 0·05 ml. weekly, till at the eighth injection 0·5 ml. is given. If a second course of injections is needed, it is probably wise to rest the patient for a month or two. There seems to be fairly general agreement now that toxoid is of considerably more value than vaccines of whole staphylococci. Furunculosis and staphylococcal skin lesions appear to be particularly benefited by toxoid treatment. The results have been disappointing in chronic osteomyelitis (Blair and Hallman 1936, Buchman 1937). In cases of acne, staphylococcal toxoid may well be combined with a vaccine of *C. acnes*. (For reports on this method of treatment see Dolman 1933, 1935, Dolman and Kitching 1935, Connor and McKie 1934, Parish, O'Meara, and Clark 1934, Murray 1935, Whitby 1936, Ramon *et al.* 1936, Mercier 1937.)

Vaccines of *Bact. coli* and of *Proteus vulgaris* are sometimes of use in urinary infections. For further discussion of the value of vaccine treatment the reader is referred to the monograph by Dudgeon (1927.)

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CHAPTER 68

THE BACTERIOLOGY OF RHEUMATIC INFECTIONS AND OF ENDOCARDITIS

THE attempts which have been made to solve the problem of the ætiology of acute and chronic rheumatism are legion, and the literature is correspondingly extensive. An adequate review is impossible, and would indeed serve no useful purpose; for the whole subject is at the moment too confused and obscure to allow of any systematic presentation. It may be said that, while most of the earlier observations that yielded positive results tended to inculpate one or another type of streptococcus, the present position of this problem is that interest is focussed on the relation of hæmolytic streptococci to acute rheumatic infection. The earlier studies have, for the moment at least, mainly a historical interest, and may therefore be very briefly summarized.

Westphal, Wassermann and Malkoff (1899) isolated a streptococcus from the blood of a patient suffering from rheumatic endocarditis associated with chorea. In the following year Poynton and Paine (1900, 1913) published the first of their series of papers, in which, on the basis of extensive bacteriological studies in man and numerous experiments on the rabbit, they reached the conclusion that acute rheumatism is the result of infection with a particular variety of streptococcus, or with a closely allied group of streptococcal strains. Since the appearance of their reports, the bacteriologist has been mainly concerned with repeated attempts to determine the real nature of the association between certain types of streptococci and this particular disease. The observations of Poynton and Paine, with regard to the isolation of streptococci from the blood, exudates, or tissues of patients with acute rheumatism have been repeatedly confirmed (Beaton and Walker 1903, Walker and Ryffel 1903, Loeb 1908, Camisa 1910, Beattie and Yates 1912, Rosenow 1914, Lyall 1914, Swift and Kinsella 1917, Quigly 1918, Clawson 1925, Birkhaug 1927, Small 1927, Zinsser and Yu 1928, Kreidler 1928). The distribution of these streptococci in the body is not, however, such as one would expect on the assumption that the rheumatic manifestations are the result of a bacteræmic infection, associated with, or followed by, a localized proliferation in particularly susceptible tissues. In very few instances have the streptococci been cultivated from the joint fluid in acute rheumatic arthritis. Isolation from the blood during life has been successful in a somewhat larger proportion of cases; but a considerable number of the strains described by various authors have been cultivated at autopsy, from the blood, from the pericardial fluid, or sometimes from the spleen. Moreover, the presence of streptococci in the blood, exudates or internal tissues is by no means a constant feature of acute rheumatic infection. In a large proportion of cases cultures obtained during life remain uniformly sterile, and careful bacteriological examinations carried out at autopsy on patients who have died from an acute attack may give entirely negative results (Bulloch 1912). The report by Cecil, Nicholls and Stainsby (1929*a*, *b*) that streptococci could be isolated from the blood of a high proportion of rheumatic patients by the use of a special technique gave a fresh impetus to

work along these lines; but there has been an almost entire failure to confirm their results (see Nye and Seegal 1929, Nye and Waxelbaum 1930, Margolis and Dorsey 1930, Cooley 1932, Dawson, Olmstead and Boots 1932*a*, Lichtman and Gross 1932, Steinfeld 1932).

To summarize: it is seldom possible to cultivate any bacteria from the local lesions during life; blood cultures remain sterile in a large proportion of cases during the febrile stage of the disease; but, in either case, when any bacterium has been isolated, it has almost invariably been a streptococcus; cultures obtained at autopsy have, as would be expected, given growth in a higher proportion of cases, though by no means in all, and here again a streptococcus has been the predominating organism, either in pure culture or in association with those bacteria which occur as common, non-specific contaminants of cultures taken under these conditions.

With regard to the particular type of streptococcus concerned, the great majority of the strains isolated have been definitely described as non-hæmolytic, that is, as not giving rise to true hæmolysis. In many cases, probably in the majority, the organism has been identified as *Str. viridans* by the green coloration given on blood agar; in others, and particularly in some recent reports, the strains described are recorded as being without action on red blood corpuscles.

From the experiments of Poynton and Paine onwards, the study of the lesions produced in rabbits by the intravenous inoculation of streptococci has played a large part in the bacteriological attack on the problem of rheumatic infection. The results of these extensive series of observations may be very shortly summarized. Many of those lesions which are characteristic of rheumatic infection in man have been reproduced in the rabbit as the result of such inoculations. The experimental lesions include endocarditis, non-suppurative myocarditis, and arthritis of all degrees of severity, sometimes associated with extensive, secondary bony changes (Poynton and Paine 1900, 1913, Cole 1904, Wächter 1908, Bracht and Wächter 1909, Coombs *et al.* 1912, Jackson 1912, Rosenow 1914, Topley and Weir 1921, Birkhaug 1927, Small 1927, Gross *et al.* 1929, Clawson 1930, Moon and Stewart 1931). But although the analogy between experimental rheumatism in the rabbit and the natural infection in man is very close, it is not exact. This applies particularly to the myocardial lesions. Aschoff (1904) described in detail characteristic nodular collections of cells in rheumatic myocarditis, and his observations have been fully confirmed by later workers (Geipel 1905, 1909, Fraenkel 1912). In spite of a certain resemblance between some of the myocardial lesions in rabbits and the typical Aschoff nodule (Coombs *et al.* 1912, Jackson 1912), it is very doubtful whether the rabbit lesions can be regarded as pathognomonic (Wächter 1908, Bracht and Wächter 1909, Topley and Weir 1921, Gross *et al.* 1929).

However closely experimental streptococcal infection in the rabbit simulates acute rheumatism in man, it is quite certain that the ability to produce these lesions on intravenous inoculation cannot be regarded as evidence that a particular variety of streptococcus is ætiologically related to the human disease; for many of the streptococcal strains which have produced the most characteristic lesions in the rabbit have been derived from sources quite unconnected with rheumatic infection, and some of them have been actively hæmolytic (Cole 1904, Jackson 1912, Topley and Weir 1921). It is clear that experimental rheumatism in the rabbit is a characteristic reaction of that animal species to the intravenous inoculation of a variety of different streptococci.

Before leaving the problem of the possible relation of these heterogeneous strains of streptococci to rheumatic diseases it should be noted that many observers have suggested the possibility that some forms of rheumatism, at least, are allergic manifestations. This view has, for instance, been developed by Zinsser and Yu (1928) and it receives some support from the relative frequency of allergic skin

reactions to killed cultures of streptococci of various types (see, for instance, Swift, Wilson and Todd 1929).

Hypersensitiveness to the antigens of various streptococci is also common in non-rheumatic subjects (Derick and Fulton 1931), and on this basis it is difficult to relate the allergic state to rheumatic fever. Other observers (see Green 1942*d*) have found that rheumatic subjects are more frequently sensitive to extracts of streptococci and particularly of *Str. pyogenes* than non-rheumatic subjects are. Thus Gibson, Thomson and Stewart (1933) recorded a higher proportion of skin reactions to extracts of hæmolytic streptococci among 140 rheumatic cases than among 145 controls, whereas there was no significant difference between the skin reactions of these groups to extracts of non-hæmolytic streptococci. On the other hand the Dick reaction was positive in only 16 per cent. of the rheumatic cases as compared with 28 per cent. of the controls. The test extracts contained protein material, but the nature of the responsible antigen is not known. The greatest number of positive reactions was obtained in tests with extracts of autogenous streptococci, which suggests some type-specificity; but mixed extracts from several *Str. pyogenes* strains also gave a large number of positive reactions (Green 1938*a*); and the reactivity of extracts from other streptococcal species indicates the possibility of hypersensitiveness to an antigen common to them all.

It will be recalled that serum sickness in man is often associated with minor rheumatic manifestations.

Much of the confusion in regard to this problem is without doubt due to the difficulty of defining, or recognizing, rheumatism as a clinical syndrome, or series of syndromes. We have, in the previous discussion, been mainly concerned with acute rheumatic fever, where the clinical diagnosis is not in doubt, and it is to the ætiology of this condition that the following sections refer.

The Rôle of Hæmolytic Streptococci in Acute Rheumatism.—The possibility that acute rheumatism is one of the manifestations of infection with hæmolytic streptococci was raised in a definite and categorical form by the studies of Coburn (Coburn 1931, 1936, Coburn and Pauli 1932*a*, *b*, *c*, 1935*a*, *b*, *c*, *d*, *e*, *f*, *g*).

The starting-point of these studies was the observation of a close epidemiological relationship, in place and time, between hæmolytic streptococcal infection of the throat and the incidence of acute rheumatic infection. Coburn (1936) identifies three phases in the pathogenesis of the disease; a phase of acute infection of the upper respiratory tract, a second phase in which there is complete absence of signs and symptoms, and a third phase of the rheumatic manifestation proper. It was also found that, in individual cases, a relapse was frequently associated with the reappearance of hæmolytic streptococci in the throat in large numbers. In instances in which this sequence was particularly studied, it was found that acute rheumatic symptoms tended to develop 1 to 5 weeks after the original appearance of hæmolytic streptococci in the throat.

These observations have been amply confirmed by other workers. Thus Collis (1931) notes that an outbreak of tonsillitis in a ward occupied by children suffering from rheumatic heart disease led to a relapse in 9 of 11 children who developed this throat infection and from whose throats a hæmolytic streptococcus was isolated; and Glover and Griffith (1931) describe outbreaks of tonsillitis in schools due to hæmolytic streptococcal infection in which a proportion of the infected boys developed all the symptoms of acute rheumatic fever (see also Schlesinger 1930, Sheldon 1931, Thomson 1934, Green 1942*a*, *b*, *c*). The association between tonsillitis and acute rheumatism is, as Glover and Griffith emphasize, quite an old observation

in its purely clinical aspect, and Newsholme in 1895 called attention to the similarity in epidemiological behaviour between rheumatism, erysipelas, puerperal fever and scarlet fever. As in the case of scarlet fever, the effect of recent studies has been to add precision to this observation by establishing the fact that the true relationship is between acute rheumatism and infection with hæmolytic streptococci, which have proved to be *Str. pyogenes* of Lancefield's Group A (see Pauli and Coburn 1937).

Thus Green (1938*b*) records that of 200 rheumatic subjects admitted to hospital, 50·5 per cent. carried Group A streptococci in the throat, irrespective of any signs of throat infection; the rate in 200 non-rheumatic patients was only 12·5 per cent., although the incidence of a recent history of throat infections in both was approximately the same. An even closer association of the disease with *Str. pyogenes* was demonstrated by Green (1939), who cultivated it from the valvular lesions, but not from the blood, in 8 of 9 fatal cases of rheumatic fever; in the cadavers of 22 non-rheumatic subjects, *Str. pyogenes* was found on the valves only twice, and in these two cases blood cultures were also positive. Collis (1939) examined only rheumatic cases, and found hæmolytic streptococci in 14 of 15 tonsils, 13 of 27 cervical or mediastinal lymph nodes, and 22 of 42 heart valves cultured (see also Thomson and Innes 1940). In the Canadian army about 4 per cent. of persons suffering from streptococcal infection of the respiratory tract are said to have developed acute rheumatism. Of throat cultures taken from 46 patients, 14 yielded no streptococci, 2 yielded *Str. viridans*, and 30 yielded hæmolytic streptococci, all of which, with one possible exception, belonged to Group A (Feasby 1944).

The significance of the epidemiological findings is greatly increased by serological evidence of infection by *Str. pyogenes*. Todd (1932) showed that the acute rheumatic process is almost constantly associated with a wide fluctuation in the titre of antibody to the O streptolysin in the blood of the patient. This fluctuation is of a rather peculiar kind, in that the rise in titre occurs during the acute rheumatic attack, and particularly during a relapse, while quiescent or recovered cases tend to show titres that are not greatly above the normal. These observations have been confirmed by Coburn and Pauli (1932*c*, 1935*a*, *c*, *f*).

Later observations showed that the streptolysin O titre rose in scarlet fever and streptococcal pharyngitis. The response in these two diseases is at a maximum two to three weeks after their onset; that in rheumatic fever occurs rather later—4 weeks or more after the onset (Coburn 1936, Coburn and Pauli 1939*b*, Todd, Coburn and Hill 1939). Green (1941) gives 79 units per ml. as the average titre in healthy subjects, 263 in pharyngitis, 300 in scarlet fever, and 210 in inactive, and 444 in active rheumatic fever.

There is other evidence of a serological response to the antigens of *Str. pyogenes*. Agglutinating and complement-fixing antibodies for hæmolytic streptococci (Coburn and Pauli 1932*c*), antifibrinolysin (Stuart-Harris 1935, Lichty 1941, Mote and Jones 1941), and precipitins for group and type specific antigens of Group A streptococci (Swift and Hodge 1936, Coburn and Pauli 1939*b*, Mote and Jones 1941) resemble anti-streptolysin O in occurring later in rheumatic fever than in other streptococcal infections.

The antibody to the streptolysin S (see Chapter 24) is an exception to this generalization, for though raised in patients with streptococcal infection and rheumatic fever, in the rheumatic subject it is depressed in the acute stage, though not to normal levels (Todd, Coburn and Hill 1939). It should be noted that relatively high antibody levels may be found in apparently healthy persons with no history of recent infection by streptococci (Solomon 1943): and that the correlation between antibody response and rheumatic fever is not complete; in a small number of cases typical rheumatic attacks may take place without change in antibody titre.

There are many indications that an important genetic factor is concerned, and that certain individuals have a special tendency to respond to infection with hæmolytic streptococci in this particular way (see Green 1942*c*, Wilson, Schweitzer and Lubschez 1943).

In summary, the evidence that hæmolytic streptococci play a significant part in the pathogenesis of acute rheumatic infections is strong, but it is by no means complete. An initial streptococcal pharyngitis is common, but may be absent in a few cases. In recent years, hæmolytic streptococci have been found in rheumatic lesions, but not in all cases; these results are in marked contrast to those of earlier workers. There is further support for the rôle of hæmolytic streptococci in the fact that the prophylactic administration of sulphonamides to rheumatic subjects has, in a number of controlled tests, apparently reduced the incidence both of streptococcal infections of the upper respiratory tract, and of the rheumatic recurrences that usually follow them (Coburn and Moore 1939, 1941, Thomas 1942, Hansen, Platou and Dwan 1942, Kuttner and Reyersbach 1943, Feldt 1944). The serological response to various antigens of hæmolytic streptococci is also suggestive, but here again it is not an invariable concomitant of rheumatic fever. From the lack of an antitoxin response in the rheumatic subject, and from the failure of immunization with *Str. pyogenes* toxin to prevent the development of acute rheumatism (see Coburn and Pauli 1935*a*), we may conclude that the erythrogenic toxin plays little part in the disease process. The probable participation of other soluble antigens is suggested by the reports of a reduction of rheumatic recurrences in children immunized by repeated doses of filtrates prepared from cultures of hæmolytic streptococci (see Wasson and Brown 1942).

Even though we accept the broad indications of the streptococcal rôle in acute rheumatism it is clear that, compared with typical infections like pharyngitis or scarlet fever, the rheumatic response to the streptococcus is curiously delayed. The cause of this abnormality does not appear to lie in the organisms themselves, for they do not demonstrably differ from strains associated with other types of infection. Poverty, bad housing, urbanization, dietary deficiences and hereditary tendencies have all been implicated as predisposing factors (see, for example, Glover 1939, 1943, Morris and Titmuss 1942), but are not easily interpreted in terms of a peculiar reactivity to *Str. pyogenes*. As we have seen in Chapter 54, there is little basis for implicating vitamin C deficiency as a major factor (see also Keith and Hickmans 1938).

We may justifiably invoke allergy to explain some features of the disease; but the question as to why a proportion of the persons infected with hæmolytic streptococci should react in this peculiar manner remains unanswered. Coburn (1940) suggests that the delayed reaction to the initial streptococcal infection is due to an inadequate response of the normal defence mechanisms, which permits the establishment of streptococcal foci in the tissues. The liberation of antigen from these foci results at first in allergic sensitization, and, at a later stage, in a concurrent stimulation of the antibody-forming apparatus and in acute allergic inflammation in other sensitized tissues. The observation of Coburn and Pauli (1939*a*) that, in the quiescent stage after the initial pharyngitis prior to the onset of the rheumatic attack, a substance ("precipitinogen") appears in the blood, which is specifically precipitated by sera taken from the same patient or from other patients in the later acute rheumatic stage, suggests the replacement of a circulating antigen by an antibody specific for it, and to this extent at least offers additional support

for Coburn's view. The "precipitinogen," however, has not yet been identified with any known streptococcal antigen.

The Virus Hypothesis.—Alternatively, we may postulate another infecting agent as responsible, either in conjunction with hæmolytic streptococci, or independently of them.

Schlesinger, Signy and Amies (1935) found that the deposits obtained by high-speed centrifugation of specimens of pericardial fluid from cases of acute rheumatism contained bodies which, in their size and general morphology, closely resembled virus particles. Relatively pure suspensions of these particles in formol saline were agglutinated by sera from patients suffering from, and successfully resisting, an acute rheumatic infection, while they were not agglutinated by normal sera, or by sera obtained from patients suffering from non-rheumatic infections. The authors did not dispute the importance of hæmolytic streptococcal infections in relation to acute rheumatism; but concluded that such infection in some way lowered resistance to invasion with the virus, or reactivated it if it was already present (see also Schlesinger, Signy and Payne 1935).

Subsequent workers were unable to confirm the virus-like nature of these bodies or the specificity of their association with acute rheumatism. The bodies were present, but not consistently, in the cerebrospinal fluid of patients with chorea, and the joint fluids in rheumatoid arthritis (Eagles *et al.* 1937).

The formation of agglutinins for these bodies, moreover, was found to be associated with arthritis, whether rheumatic, rheumatoid, gonococcal or pneumococcal and not with the specific rheumatic state (Eagles and Bradley 1939). Attempts to reproduce rheumatism experimentally by inoculating fresh rheumatic exudates containing the bodies into the joints or the pericardial cavity of monkeys, proved fruitless (Eagles *et al.* 1938).

Rheumatoid Arthritis.—It may be noted that there is both epidemiological and serological evidence that rheumatoid arthritis is associated with hæmolytic streptococcal infection; high agglutinin, precipitin and antistreptolysin titres have been reported in patients suffering from this disease (Dawson, Olmstead and Boots 1932*b*, Dawson, Olmstead and Jost 1934, Dawson and Tyson 1936, Neil and Hartung 1937; see also Goldie 1938).

Bacterial Endocarditis

Although this disease bears no immediate or necessary relationship to acute rheumatism, it is convenient to consider it in this chapter, if only because the isolation of non-hæmolytic streptococci from the blood stream in the more chronic cases of this kind has perhaps done something to confuse the issue in regard to the causation of simple rheumatic endocarditis.

The bacteria most commonly found in large proliferating and ulcerating lesions of the heart valves are the various pyogenic cocci: hæmolytic streptococci, streptococci of the *Str. viridans* and *Str. fæcalis* types; pneumococci, gonococci and meningococci; *Staph. aureus*, *Staph. albus* and micrococci of various kinds. Of other bacteria, bacilli of the *Hæmophilus* group occur with the greatest frequency (see Perry 1936), though endocardial infections with members of the *Bacterium* and *Bacillus* groups, with salmonellæ, brucellæ, corynebacteria, clostridia (see More 1943) and so forth, have occasionally been reported.

In a number of instances, the endocarditis is apparently secondary to infection elsewhere in the body, usually by the more pathogenic organisms like *Staph. aureus*, *Str. pyogenes* or the pneumococcus. In others, the infection of the heart valves

is apparently primary, and usually runs a less acute, though no less fatal course than that in the first group.

Subacute Bacterial Endocarditis.—There can be no question that this fatal disease is due in the great majority of cases to the infection of a previously damaged, or congenitally abnormal, valve with streptococci, either of the viridans or of the enterococcus type (see Moran 1938, Swain 1940, Solowey, 1942), though other organisms, including influenza bacilli and *H. para-influenzæ*, may occasionally be concerned (see, for instance, Horder 1908–9, Schottmüller 1910, Kreidler 1926, Stuart-Harris *et al.* 1935, Miles and Gray 1938, Khairat 1940).

Our knowledge of the pathogenesis of this condition was greatly advanced by the important studies of Lewis and Grant (1923), Grant (1924), and Grant, Wood and Jones (1928) on the pathological side, and by Okell and Elliott (1935) in regard to the probable source and mechanism of infection.

Lewis and Grant (1923) found that 26 per cent. of an unselected series of cases of subacute bacterial endocarditis had a congenitally bicuspid aortic valve; and they calculate, on the basis of their experience, that of these persons with congenitally bicuspid aortic valves who reach adult life, some 23 per cent. die of subacute endocarditis. Grant (1924) and Grant, Wood and Jones (1928) described the lesions of this disease in considerable detail, and gave reasons for believing that they are usually produced by direct infection of the endocardium from the blood stream, and not from embolism of the smaller vessels, where these are present in the endocardial and sub-endocardial tissues attached. In fact, the essential predisposing lesions, in which the circulating bacteria establish themselves, appeared to be small platelet thrombi on the valvular surfaces. Allen and Sirota (1944) concur in finding predisposing lesions, but consider that their essential feature is a degenerative process primarily involving collagenous tissue. On the other hand, McNeal, Spence and Slavkin (1944), in their study of experimental streptococcal endocarditis in the rabbit, report that circulating bacteria are ingested by the endothelial cells of blood vessels all over the body, including those covering the valves of the heart. The valves become infected because, unlike the rest of the vascular endothelium, that on the valves is not bactericidal, and is consequently damaged by the ingested cocci. The injury is then covered by a deposit of fibrin, which, by protecting the ingested streptococci, enables them to set up a local infection.

Okell and Elliott (1935), approaching this problem from a different angle, endeavoured to find a source from which the infecting streptococci might be derived. Before extraction of the teeth, they found streptococci in the blood of 12 of 110 patients suffering from moderate or extensive gum disease, but in none of 68 persons with healthy gums. After extraction, the incidence of positive blood cultures rose, but the bacteræmia observed was transitory, since the blood became sterile, often within ten minutes of becoming infected. Transient bacteræmia of this kind occurred in 72 of 100 patients with gum disease, and in 12 of 38 patients without obvious gum disease. The majority of cocci isolated were of the *Str. viridans* type. These findings have been amply confirmed (for references see Pressman and Bender 1944); transient bacteræmia may follow even light manipulation of infected teeth. The same sequence has been observed after operative manipulation of other infected sites—the cervix uteri (Schottmüller 1911), the urethra (Barrington and Wright 1930), joints, tonsils and prostate (Richards 1932), the organism found depending on the nature of the infection. Tonsillectomy

approaches tooth extraction in the frequency with which it is followed by a *Str. viridans* bacteræmia (see Fischer and Gottdenker 1936, Elliott 1939).

It would seem, therefore, that in any person with oral or tonsillar sepsis, there is likely to be an occasional leak of streptococci into the blood stream, leading to a transitory bacteræmia, this leak being temporarily intensified as the result of operative procedure. These streptococci are, in a normal person, rapidly removed from the blood stream and cause no serious damage to the tissues; but if they come in contact with a congenitally defective heart valve, or a valve already damaged as the result of rheumatic infection, or a valve in which degenerative changes have occurred, they may set up a lasting and slowly fatal infection.

There are recent reports of the cure, by both sulphonamide compounds and penicillin, of this normally fatal disease. Heparin is sometimes given as well, to prevent the deposition of fibrin on the surface of the infected lesions during treatment (see Loewe *et al.* 1944). The data, however, are as yet too scanty to permit any conclusion about the value of chemotherapy. With regard to prophylaxis, attempts have been made to minimize the risk of the bacteræmic showers induced by manipulation of infected lesions in the body. Pressman and Bender (1944), for example, report that sulphanilamide, though it does not reduce the incidence of the transient bacteræmia following tooth extraction, appears to hasten the subsequent sterilization of the blood stream.

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CHAPTER 69

ENTERIC INFECTIONS

Among the clinical records left by medical writers from Hippocrates onwards, we have little difficulty in recognizing cases which can, with reasonable certainty, be identified as instances of enteric fever. The separation of this type of infection from the mass of continued fevers was, however, a slow process; and it was only during the first half of the nineteenth century that typhoid or enteric fever finally emerged as a clinical syndrome from among the mass of continued fevers with which it had previously been confused. Various differences in behaviour between typhoid fever and the prevalent typhus, gaol, or famine fever had indeed been noted at much earlier dates. Among these pioneers were Willis (1659) and Huxham (1739); but their descriptions were not sufficiently detailed or complete to carry general conviction. As noted by Creighton (1894), the final recognition of enteric fever in this country resulted from the elaborate analysis of the symptoms of the different types of continued fever carried out by Sir William Jenner between 1849 and 1851. In Germany, the differentiation of typhus from typhoid was clearly recognized by Schoenlein (1839), under the names "Typhus exanthematicus" and "Typhus abdominalis," which have maintained their position in German literature. In France, the observations of Prost (1804), and particularly of Petit and Serres (1813), afforded what was probably the first accurate description of the intestinal lesions; though many earlier records of post-mortem findings are in existence. These observations were confirmed and extended by Bretonneau (1826), Louis (1829), and Chomel (1834)—(see Gay 1918).

The most striking contribution to our knowledge of the natural history of typhoid fever, before the opening of the bacteriological era, is undoubtedly that made by William Budd (1856, 1873) of North Tawton in Devon. Budd had studied under Louis at the La Pitié hospital, and was therefore in a position to identify typhoid fever with considerable confidence. He insisted on its spread by contagion, on the fact that the infective agent was excreted in the fæces, on the rôle played by the contamination of water and milk in the epidemic spread of the disease, and on the possibility that infection might be derived from the excretions of a convalescent patient. His writings afford an admirable example of the value of accurate observations, carried out in the spirit of the field naturalist.

With the description of the typhoid bacillus by Eberth in 1880, and its isolation by Gaffky in 1884, it became possible to attack the problems of enteric infection by the methods devised by the bacteriologist. The subsequent isolation and study of the various species of paratyphoid bacilli showed that enteric fever, though a clinical syndrome, might result from infection with several distinct, though nearly related, bacterial species. For a description of these species reference may

be made to Chapter 30. The studies of recent years have yielded a considerable mass of data with regard to the distribution, in place and time, of the various bacterial types of enteric infection; and this point will be discussed later in this chapter. For the moment we may consider the results obtained in the detailed study of typhoid infection, in man and animals, taking them as representative of the behaviour of enteric infection as a whole, and noting certain exceptions for purposes of comparison or illustration.

Bacteriology.

During an attack of typhoid fever, the causative bacilli can be isolated from the fæces (Pfeiffer 1885), from the urine (Hueppe 1886), from the rose spots of the eruption (Neuhaus 1886), and from the blood (Vilchur 1887). It may be noted that the intensive study of enteric infection in particular samples of the population, to which we shall refer later, has resulted in the demonstration that *Salm. typhi* may occasionally be cultivated from the fæces during the incubation period, or even from the blood of an apparently healthy person, who may soon after develop the disease (Conradi 1907, Mayer 1910). The demonstration of specific agglutinins in the blood, usually attributed to Widal (1896) (since Grünbaum (1896), who noted their appearance earlier, recorded his observations later), has made it possible to study the progress of this particular response to infection, and to correlate the results with those obtained in attempts to isolate the causative organism.

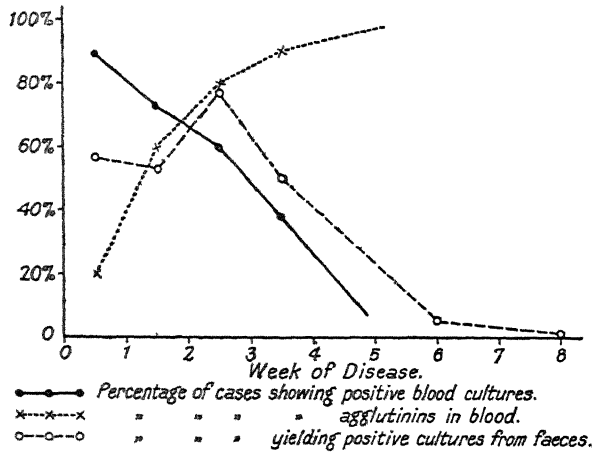


FIG. 283.

A very instructive picture of the usual course of events during an attack of typhoid fever may be obtained by charting, for the successive weeks of the disease, the average frequency with which *Salm. typhi* can be isolated from the blood, or from the fæces, and the frequency with which the serum gives the diagnostic agglutinin reaction. In preparing Fig. 283 along these lines, we have employed for the fæces the figures of Gaehtgens and Brückner (1909), extrapolating by taking values beyond the 4th week intermediate between those recorded by Semple and Greig (1908), and those suggested by the findings of Lentz (1905); for the blood the figures recorded by Coleman and Buxton (1907), again extrapolating beyond the 4th week; and for the agglutinins the figures given by Park and Williams (1925).

As indicated in the figure, the onset of symptoms is associated with a bacteræmia. From the 1st week onwards the frequency with which *Salm. typhi* can be isolated from the blood falls, at first slowly, then more rapidly. By the

end of the 3rd week it can be found in about half the cases; after the 4th week its isolation is infrequent. It must be remembered that we are dealing with a frequency chart, not with the course of events in any individual case. It may be taken that the frequencies noted for the results of blood culture after the 4th week refer to cases which were febrile at that period, since such patients would naturally be selected for investigation. In any given case, it will usually be found that the decline of fever is associated with the disappearance of bacteria from the blood. In cases which terminate fatally as the result of the primary infection, apart from the secondary accidents of hæmorrhage and perforation, the severity of the bacteræmia usually increases until death (Jochmann 1914).

The frequency of *Salm. typhi* in the fæces, on the other hand, increases from the 1st to the 3rd week and then falls somewhat slowly. The frequency curves for blood and fæces cross somewhere about the end of the 2nd week. Before that time, the bacilli are isolated more frequently from the blood than from the fæces; after it, the position is reversed. In attempting deductions from such figures as these, we must remember that differences in technique may have an important influence on our results. There are certain technical difficulties associated with blood culture, and with the isolation of pathogenic bacteria from the fæces; but they are different in kind, and the technical error involved may be quite different in degree. The significant point is the decreasing frequency of detectable bacteræmia, and the increasing frequency of *Salm. typhi* in the intestine, during the first 2 to 3 weeks. This clearly suggests that the main direction of invasion during this period of the infection is from the blood stream to the intestine, not from the intestine to the blood. In the 1st week about 20 per cent. of the cases give a positive agglutination test. The curve then rises sharply, crossing the blood-culture curve just before the end of the 2nd week, and still rising attains a value of 90 per cent. or more by the 4th week; after which it remains at a high level for several weeks.

Data are available which enable us to describe with considerable accuracy the course of the agglutination curve in an individual case of typhoid fever, plotting titre against time. The most extensive records, however—those of Dreyer and his colleagues—refer to results obtained with formolized broth cultures, which react chiefly to the H (flagellar) agglutinins (Dreyer *et al.* 1915, 1916, 1917). We have no such detailed information for the rise and fall of O (somatic) agglutinins; but such evidence as is available suggests that the general form of the curve, at least over the earlier part of its course, is very similar to that followed by the H agglutinins. This question will be discussed more fully in relation to diagnosis. For our immediate purpose we may take it that agglutinins usually appear in the blood between the 3rd and 7th day of disease, rise, first slowly, then more steeply to a maximum between the 16th and 22nd days, and then fall, at first steeply, later (at least in the case of the flagellar agglutinins) very slowly, so that they are detectable for weeks or months after convalescence (Figs. 260, 261, p. 1121).

The rise in agglutinins is usually associated with a decreasing bacteræmia, and the time at which the agglutinin titre reaches its maximum coincides approximately with an amelioration in the patient's general condition, as indicated by a gradual fall in temperature. In this connection the agglutinin curve, so far as it is described in terms of H agglutinins, should be regarded as an indication of a general antibody response, not as deriving its significance from the fluctuation in flagellar agglutinins themselves. There are many reasons for believing that these particular antibodies

have little if any protective value, while the O agglutinins, and apparently the Vi antibody of Felix and Pitt (1934*b*), are of decisive importance from this point of view (see Chapter 47 and later sections of this Chapter).

As Fig. 283 indicates, the bacilli do not disappear from the intestine so quickly, or so completely, as from the blood. There is a lag, which is relatively constant over the early part of the falling frequency curve, but which becomes more pronounced later. A considerable proportion of cases are still excreting *Salm. typhi* in their fæces at a time when positive blood cultures can no longer be obtained; and some cases continue excreting well into convalescence or beyond it.

The results obtained in an extensive series of investigations, at the bedside, on post-mortem material, and by direct experiments on animals, have indicated the probable sequence of events during an attack of enteric fever.

If cultures are taken from various situations during a post-mortem examination, the distribution of typhoid bacilli is usually found to be as follows. In the intestine itself almost pure cultures are frequently obtained from the duodenum and upper part of the jejunum. At lower levels the bacilli become less and less numerous in proportion to the other intestinal bacteria; and, below the ileo-cæcal valve, they may be so outnumbered by *Bact. coli*, and by other organisms, as to escape detection in artificial culture (von Drigalski 1904). Outside the intestine, there are four situations in which *Salm. typhi* is almost constantly present, and from which it can often be isolated in pure culture: the spleen, the enlarged mesenteric glands, the bone marrow, and the gall-bladder. It has been pointed out by Levy and Gaehtgens (1908) that, while the mesenteric glands are always enlarged and cultures from them almost constantly positive, other glands are frequently unaffected. It is of interest to note (Mallory 1898) that the enlargement of the mesenteric lymph nodes is associated with marked proliferation of the endothelial cells of the lymph sinuses, while the lymphoid cells are but little affected.

The intravenous inoculation of typhoid bacilli into rabbits or guinea-pigs is followed by their deposition in the liver, spleen, lymph glands, and bone marrow; but there is, in this case, no selective localization in the mesenteric glands. Lüdke (1909) noted the persistence of typhoid bacilli in the lymphatic glands and bone marrow of the guinea-pig, following intravenous inoculation, and their proliferation in these situations after their disappearance from the blood. Gay (1918) finds that typhoid bacilli persist in the bone marrow of infected rabbits after they have disappeared from the blood, lymph nodes and spleen. Reference to Chapter 47 will afford additional data with regard to the fate of typhoid bacilli after intravenous or intraperitoneal inoculation (Buxton 1907*a*, *b*, Bull 1915).

The presence of typhoid bacilli in the gall-bladder is of particular interest, as will later appear. Von Fütterer (1888) was apparently the first to demonstrate that *Salm. typhi* could be isolated from the gall-bladder in fatal cases of the disease. Gilbert and Girode (1890) confirmed its presence in this organ and Chiari (1894) noted its frequency. Pratt (1901) isolated *Salm. typhi* from the gall-bladder in 21 of 30 fatal cases of typhoid fever, and Scott (1915) from 24 of 28 cases. The evidence for the occasional persistence of typhoid bacilli in the gall-bladder long after convalescence is equally conclusive. Droba (1889) isolated *Salm. typhi* from the gall-bladder contents, and from the interior of the gall-stones removed at operation 17 years after an attack of typhoid fever. Findlay

and Buchanan (1906) isolated the bacillus from the gall-bladder 25 years after an attack, and at the same time demonstrated it in the fæces. In some cases, as in that reported by Dudgeon (1908), *Salm. typhi* has been isolated from the gall-bladder in a case of cholelithiasis, in the absence of any history of typhoid fever. The literature contains many instances of a similar kind (see Ledingham and Arkwright 1912, Gay 1918).

The mechanism by which the gall-bladder becomes infected has been studied experimentally in rabbits. Blachstein (1891) found that bacilli could be isolated from the gall-bladder of these animals for days, or weeks, after an intravenous inoculation of living culture. Richardson (1899) recorded similar observations, and noted that some of the rabbits developed gall-stones, an observation which has been repeatedly confirmed (Gay 1918). The bacilli may reach the gall-bladder very rapidly after intravenous injection, according to Blumenthal (1910) within 10 minutes. They may persist in this situation over long periods (Morgan 1911, Johnston 1912, Gay and Claypole 1913). Weinfurter (1915) records an instance in which they were still present after 9 months.

The most generally accepted view, as regards the route by which the bacilli reach the gall-bladder from the blood, is that they travel from the liver capillaries to the bile canaliculi, and thence down the bile ducts (Doerr 1905, Lemierre and Abrami 1907, Nichols 1916). It has, on the other hand, been stated (Koch 1909, Chiarolanza 1909) that they rapidly reach the gall-bladder even after ligation of the cystic duct, and that nests of bacilli may be found in the capillaries of the gall-bladder wall, suggesting that they pass directly into the viscus by this route. It is probable, as suggested by Gay (1918), that either route may be traversed.

The picture that we have just given of the distribution of typhoid bacilli in the blood, fæces, and tissues is based on results obtained by a technique which, in respect of fæces at least, we know to be considerably inferior to that now at our command. It may well be that the frequency of typhoid bacilli in the intestine during the early stage of the disease has been considerably underestimated. That this is true of paratyphoid B fever, the observations of Glass and Wright (1937) leave no doubt. Using a combination of tetrathionate broth for preliminary enrichment, followed by plating on eosin brilliant green agar, these workers were able, during the investigation of a sharp outbreak of paratyphoid fever at Liverpool, to isolate the bacilli from the fæces of about 83 per cent. of patients in the first week, 94 per cent. in the second, and 88 per cent. in the third. Had more than one specimen of fæces been examined from each patient, the proportion of positive results might have been even higher. Whether the same holds true of typhoid and of paratyphoid A fever is not known; observations on these two diseases by the improved techniques of isolation that have been developed of recent years are badly needed. It is interesting to note that, according to Glass and Wright (1937), paratyphoid B bacilli are not likely to be isolated from the urine during the first six weeks of the disease unless the fæces are also positive; after this time, however, during the so-called "clearance period," the urine is not infrequently positive when the fæces have become negative. Gell and Knox (1942), in their study of the Kettering outbreak of paratyphoid B fever, reached the same conclusion.

Route of Infection.—We have not yet considered the question of the route by which the bacilli gain access to the tissues and the blood stream, during the initial stages of naturally occurring infection. Our knowledge of the epidemiology of enteric fever, and of its mode of spread, makes it quite clear that the bacilli enter the body by the alimentary tract; the point at issue is the situation at which

penetration of the mucosa occurs. It was originally believed that the bacilli passed through the stomach, proliferated in the intestine, and thence invaded the blood stream. This view has been very generally discarded; since it seems clear that the heavy intestinal infection which occurs during the 2nd and 3rd weeks of an attack of typhoid is due, in large part, to the passage of bacilli into the intestine from the gall-bladder, and perhaps from the Peyer's patches and lymphoid follicles. Some authors (Förster 1908) insist that there is no reason to suppose that *Salm. typhi* ever multiplies within the intestinal canal. It has been suggested that the bacilli usually gain access to the tissues *via* the tonsils and lymphoid tissue of the pharynx, rather than through the intestinal wall (Brion and Kayser 1906); and von Drigalski (1904) has reported an epidemic in which 40 per cent. of the cases suffered from an initial faucial angina. In many of these cases he obtained a pure culture of *Salm. typhi* from the tonsils. Whether or not this route may be followed in some cases, there seems no adequate reason for discarding the traditional view that the usual portal of entry to the tissues is through the intestinal lymphatics; and this appears to be supported by the finding that the mesenteric glands are constantly affected.

We have already referred, in Chapter 47, to experiments recorded by Ørskov, Jensen and Kobayashi (1928), who noted the following sequence of events in experimental mouse typhoid, after infection *per os* with *Salm. typhi-murium*: the destruction, in the intestinal tract, of the greater part of the ingested bacteria, as evidenced by a transitory excretion of bacilli followed by a period during which none could be recovered from the fæces: the passage of some bacilli to the mesenteric glands, and their further passage to the blood stream, probably *via* the thoracic duct: a transitory bacteræmia, rapidly brought to an end by the removal of the bacilli by the reticulo-endothelial cells, particularly those of the liver and the spleen: a phase during which active proliferation is proceeding in the liver and spleen, as evidenced by the recovery of increasing numbers of bacilli from these organs, but during which the blood remains sterile: a phase of secondary bacteræmia, associated with a generalization of bacteria throughout the tissues, and a secondary invasion of the intestine, principally *via* the gall-bladder and bile duct.

It seems likely that a similar sequence of events occurs in natural infection in man. If so, it may well be that the incubation period in typhoid fever corresponds to the phases which precede the secondary invasion of the blood stream from the sites of primary proliferation in the liver, spleen and mesenteric glands, the occurrence of this secondary bacteræmia marking the onset of the actual illness. Such a conception receives some support from the occasional isolation of *Salm. typhi* from the fæces and from the blood during the incubation period of the disease.

We have already considered in some detail the course of events during the later stages of the illness, and the factors which determine, in some cases, the persistence of bacilli in the tissues over long periods of time, and their persistent, or intermittent, excretion in the fæces. A few points remain to be discussed. The characteristic lesions in Peyer's patches appear to result from an initial hyperplasia of the endothelial cells in response to the bacterial invasion, followed by necrosis and sloughing, caused in part by the toxic action of some bacterial product, in part by interference with the blood supply. Perforation and hæmorrhage are in the nature of occasional associated accidents. The gall-bladder is one of the most frequent sites of persistent infection; and is, from the epidemiological point of view, the most important, because of the ease with which bacilli escape thence to the intestine, to be excreted in the fæces; but it is by no means the only situation in which the bacilli may remain latent over long periods of

time. Experience with mice (Topley 1926) suggests that the spleen may form a persistent focus of infection; and the well-known typhoid periostitis affords an example of a lesion which may occur long after convalescence from the original illness.

It should be noted that relapses may occur during convalescence, although they are relatively infrequent. They are usually milder and of shorter duration than the original attack; and the mortality is low (Gay 1918). They are undoubtedly due to a re-invasion of the blood stream from the tissues in which typhoid bacilli are still proliferating at the time when the bacteræmic phase of the primary attack is brought to a close. They are milder, and of shorter duration, presumably because specific antibodies are already present, and because the tissues respond promptly to the secondary stimulus.

The Vi Antigen of the Typhoid Bacillus.

It will be convenient at this point to discuss the Vi antigen of the typhoid bacillus, since it will be necessary to refer to it under diagnosis, prophylaxis, and serum treatment of typhoid fever, as well as in relation to the bacteriophage typing of the typhoid bacillus.

Felix and Pitt (1934*a*) found that, when different smooth strains of typhoid bacilli were compared with one another in regard to their sensitiveness to agglutination by an O antiserum, they differed very widely, and that this sensitivity was inversely related to the virulence of the strains as judged by the intraperitoneal injection of mice. Thus three strains that were very slightly agglutinable by an O antiserum killed 10 of 10, 10 of 10 and 8 of 10 mice when injected in a dose of 100×10^6 bacilli; whereas three strains that were freely agglutinable by an O antiserum failed to kill a single mouse when injected in this dose. A high proportion of strains freshly isolated from cases of typhoid fever have been found to be of the mouse-virulent type. In a later paper Felix and Pitt (1934*b*) showed that the virulence of the former strains, and their inagglutinability by an O antiserum, were due to the presence of a special antigenic component, which Felix called the "Vi" antigen.

This antigen, it may be noted, is heat labile; so that agglutination tests to detect its presence or absence must be carried out with living suspensions at a temperature of 37° C. Rabbits immunized with living cultures of highly virulent typhoid bacilli produce, in addition to the usual O antibodies, a Vi antibody which agglutinates the virulent strains; while rabbits immunized with avirulent smooth strains, or with heat-killed virulent smooth strains, produce O antibodies alone. An antiserum prepared against virulent strains may be deprived of its O antibodies by absorption with avirulent bacilli, thus providing a pure anti-Vi serum. A Vi antiserum gives effective passive protection in mice against living virulent typhoid bacilli, while an O antiserum is almost or quite ineffective. An O antiserum will, however, protect mice against the toxic effects of a massive dose of killed typhoid bacilli. These observations have been extended by Felix and his colleagues in a series of subsequent experiments (see Felix, Bhatnagar and Pitt 1934, Felix and Pitt 1935, Bhatnagar 1935, Felix and Bhatnagar 1935).

As mentioned above, the Vi antigen is heat labile; judged by agglutination, it is destroyed by exposure to heat at 60° C. for 30 minutes or 100° C. for 5 minutes. Treatment with 0·5 per cent. phenol likewise destroys it. Formalin in 0·2 per cent. concentration has little immediate effect on its agglutinability, but partly denatures it, so that sera prepared by inoculation of rabbits with a formolized suspension are deficient in their protective and phagocytosis-promoting functions. Alcohol, on the other hand, destroys its agglutinability, but not its power to give rise to potent antibodies in rabbits; in horses,

however, alcohol-treated Vi suspensions call forth only a very small amount of antibody (Felix and Petrie 1938).

The observations of Boivin and Mesrobeanu (1938*a*, *b*, *c*) and of Boivin, Izard, and Sarciron (1939*a*, *b*) have shown that both the O and the Vi antigen of *Salm. typhi* consist of glycolipoids. Both are toxic to mice—the O considerably more so than the Vi. Chemically they differ in that, on acid hydrolysis, the O antigen yields about twice as much reducing sugar as the Vi, whereas the Vi antigen alone is precipitated in aqueous solutions by aluminium, uranyl, and lanthanum salts (see also Chapter 44).

The Diagnosis of Enteric Fever.

So far we have dealt with typhoid fever as the classical example of enteric infections in general. Before discussing the laboratory methods available for diagnosis we must consider briefly the question as to which members of the *Salmonella* group, other than *Salm. typhi*, give rise to a continued fever of the enteric type. In Chapter 30 we have given a summarized description of the species, or types, that fall within this group, including the type of case from which they have been isolated. Here we may confine our attention to those organisms that have been isolated, at least with some frequency, from cases of continued fever. The rôle of the various members of this group in the causation of acute gastro-enteritis (bacterial food poisoning) is considered in Chapter 72.

The organisms that are able, like *Salm. typhi*, to give rise to enteric fever are *Salm. paratyphi A*, *B* and *C* and *Salm. choleræ-suis*, particularly the *kunzendorf* variety. The reader may be reminded that in the older literature *Salm. paratyphi C* was often referred to as Hirschfeld's bacillus or as the Eastern European type of *Salm. paratyphi C* in distinction to *Salm. choleræ-suis* var. *kunzendorf*, which was often known as the Western European type of *Salm. paratyphi C*. In much of the literature the name *Salm. suipestifer* will be found in place of *Salm. paratyphi C* or *Salm. choleræ-suis*. It may be noted that the disease caused by these two species differs from that due to infection with *Salm. typhi* or *Salm. paratyphi A* or *B* in that the typical picture of an enteric infection is often complicated by lesions of a septic or suppurative type, including endocarditis (see Harvey 1937, Goulder *et al.* 1942, Schwabacher, Taylor and White 1943). It may also be noted that infection with *Salm. choleræ-suis* frequently takes the form of an acute gastro-enteritis instead of a continued fever. Among more recently identified species *Salm. enteritidis* var. *chaco*, *Salm. moscow*, and *Salm. bareilly* have been isolated from cases of continued fever.

Several other species within the *Salmonella* group, though usually causing an acute gastro-enteritis, occasionally give rise to a continued fever of the enteric type. This, for instance, is true of *Salm. typhi-murium*, perhaps better known as *Salm. aertrycke*; it is possibly true of *Salm. enteritidis*. The element of doubt is introduced by the fact that *Salm. dublin*, which is a natural pathogen of cattle, is certainly capable of causing a continued fever in man (see Smith and Scott 1930); and, since the differentiation of this variety from *Salm. enteritidis* is of recent date, it is possible that the earlier reports of isolated cases of enteric fever caused by *Salm. enteritidis* may have been instances of infection with the *dublin* species.

For our present purpose we may confine our attention to the five species that are frequently implicated in the causation of enteric fever, noting that, from this point of view, *Salm. choleræ-suis* var. *kunzendorf* appears to occupy a position of relatively minor importance. Another fact of great significance from the point of view of diagnosis is that the frequency distribution of these various *Salmonella* types is very

different in different parts of the world. This point will be elaborated in a later section.

The methods of diagnosis that are available and their relative value at different stages of the disease will be obvious from the time-relations shown in Fig. 283 and from the discussion of these relations in the text.

Cultural Methods in Diagnosis.—It should be emphasized that the actual isolation and identification of the infecting organism is always the method of choice. The organism is present in the blood in a high proportion of cases during the first week of illness and with decreasing frequency in succeeding weeks. It may be found in the fæces at any stage of the disease, and in the urine, especially in the later stages. A positive agglutination reaction may be highly suggestive, but it does not become positive till about the second week of the disease, and does not possess the same diagnostic reliability as isolation of the organism itself. The distinction between typhoid and paratyphoid fever is easy by blood culture early in the disease, but may be difficult and sometimes impossible at a later stage when diagnosis is attempted by means of agglutination tests alone.

At least 10 ml. of blood should be withdrawn with a syringe or similar device from a vein and transferred to a tube containing an anticoagulant, such as citrate, oxalate, or liquoid. On arrival at the laboratory, the blood should be transferred to a flask containing 50–100 ml. of 0·5 per cent. sodium taurocholate broth, or preferably distributed in about 3-ml. quantities into tubes containing 20 ml. of 0·5 per cent. sodium taurocholate broth, tetrathionate broth (Muller 1925), and brilliant green Esbach broth (Ruys 1934). The cultures should be incubated at 37° C. and plated out daily on to some selective medium such as the bismuth sulphite medium of Wilson and Blair (1927, 1931), the brilliant green eosin agar of Teague and Clurman (1916, 1916–17), or the desoxycholate citrate agar of Leifson (1935), and should not be discarded as negative for at least 5 days. If the only material sent to the laboratory is clotted blood, the serum should be pipetted off, and the clot, after being broken up with a sterile rod, transferred to bile salt broth. A surprisingly high proportion of successful isolations may be secured in this way; and in early cases the clot culture may be positive when the agglutination test is negative (see Soman 1932, 1934, Downie and Fairbrother 1934).

If possible, cultures from the fæces, and preferably from the urine also, should be put up at the same time as the blood. According to Glass and Wright (1937), it is rare, in paratyphoid fever at least, to find the causative organism in the urine during the first 6 weeks of the disease unless it is also present in the fæces. After that, the urine may be positive when the fæces are negative. Nevertheless, exceptions do occur (see Report 1941), and it is wise to examine both fæces and urine as a routine at any stage of the disease.

Two or three 10-ml. quantities of urine should be inoculated into liquid enrichment media and plated out after incubation on to the selective media already mentioned under blood culture.

Of late years much attention has been devoted to the isolation of typhoid, paratyphoid and other members of the *Salmonella* group from the fæces. The time-honoured method has consisted in preliminary incubation of the fæces in peptone water containing brilliant green in a final concentration of 1 : 100,000 to 1 : 500,000, followed by plating on to MacConkey's neutral red bile salt lactose agar or Endo's basic fuchsin sulphite lactose agar as a differential medium. The recent introduction of more highly selective media has, however, greatly improved the chances of isolating enteric bacilli from the stools.

Of preliminary enrichment media tetrathionate broth, either as described by Muller (1925) or in the slightly modified form of Schäfer (1935), is excellent, particularly for paratyphoid bacilli. It has the disadvantage of permitting the growth of *Proteus* bacilli—both the *vulgaris* and the *morgani* types—so that it should be used in combination with a plating medium such as brilliant green eosin agar (Teague and Clurman 1916, 1916–17, Meyer and Stickel 1918, Jones 1936) or brilliant green eosin methylene blue agar (Knox, Gell and Pollock 1942), which suppresses these organisms. Alternatively Kauffmann's (1930–31, 1935–36*a*) modification of Muller's medium, containing bile and brilliant green in addition, may be used. The brilliant green helps to check the growth of *Proteus*. A more effective medium for this purpose is said to be Ruys' (1934, 1940) brilliant green broth containing 2 per cent. of Esbach's reagent (1 per cent. picric acid and 2 per cent. citric acid), which is based on the observation of Dimitrijević-Speth (1929) that Esbach's reagent inhibits the swarming of *Proteus* bacilli; but preliminary results suggest that an even better means of suppressing *Proteus* is by the addition of cacotheline or hydroquinone to brilliant green broth as described by Jones and Handley (1945). For general purposes the sodium selenite enrichment medium described by Leifson (1936) (see Hobbs and Allison 1945) is to be strongly recommended.

Among the solid selective media the bismuth sulphite agar of Wilson and Blair (1927, 1931) has proved in the experience of most workers to be pre-eminently suited to the isolation of typhoid bacilli from fæces and sewage. For paratyphoid bacilli it is perhaps less satisfactory. Eosin brilliant green agar, studied by Jones (1936), has likewise proved its worth. Another medium that has been used increasingly of late is Leifson's (1935) desoxycholate citrate medium (see Hynes 1942). The addition of 0·5 per cent. lithium chloride to Endo's medium (Havens and Mayfield 1933) is said to improve its selectivity. In a comparison based on the isolation of 724 strains of typhoid bacilli, Mayfield and Gober (1940) cultured 74 per cent. on lithium chloride Endo, 82 per cent. on desoxycholate citrate, and 83 per cent. on Wilson and Blair's bismuth sulphite agar. In 195 instances the organism was isolated on only one medium, namely 42 on lithium chloride Endo, 65 on desoxycholate citrate, and 88 on bismuth sulphite agar.

It will be realized that if the maximum number of successful isolations is to be obtained, it is advisable to use a combination of one or two enrichment liquid media with two or three selective solid media. According to Hobbs and Allison (1945), the best two liquid enrichment media for general purposes are sodium selenite and tetrathionate, and the best two solid selective media, for plating either directly or from cultures in enrichment media, are Wilson and Blair's bismuth sulphite agar and Leifson's desoxycholate citrate agar.

Great care should be exercised over the collection of specimens of fæces and urine for examination. In hospitals imperfectly sterilized bedpans and urinals afford a frequent source of contamination, and it is much better to provide every patient from whom a specimen is required with a special grease-proof, cardboard container into which the excreta can be passed directly (see Holt, Vaughan and Wright 1942). Alternatively a rectal swab may be taken. The fæces should be examined as fresh as possible; if this is impracticable, they should be collected into the buffered glycerol saline solution described by Sachs (1939) or the desoxycholate citrate solution described by Bangxang and Eliot (1940) to prevent the overgrowth of typhoid-paratyphoid bacilli by coliform organisms. Cultures should be made on to two or three of the selective solid media just mentioned, with and without preliminary enrichment. Non-lactose-fermenting colonies may be tentatively identified by slide agglutination (Report 1939), and confirmed by motility, biochemical and tube agglutination reactions (see Bridges and Taylor 1944). It may be noted that the serum of some rabbits may contain agglutinins to the so-called α antigen, which is found in certain strains of coliform and *Proteus* bacilli. Non-lactose-fermenting colonies, not belonging to the typhoid or paratyphoid groups, may be agglutinated by

the serum of such a rabbit, and may give confusing results (Stamp and Stone 1944). The occurrence of the small colony variant of *Salm. typhi* must be remembered (see Morris *et al.* 1943).

Whatever selective and differential media are employed, the frequency of isolation of typhoid and paratyphoid bacilli will be significantly increased by making repeated platings from the primary enrichment culture. Topley and Fielden (1922) pointed out that, in an ordinary broth culture from a specimen of fæces, various bacterial species succeed one another as the dominant viable organisms, and that it is a relatively common event to obtain a pure culture of *Bact. coli* on a plate seeded after 24 hours, and a pure culture of *Salm. typhi* on a plate seeded after several days' incubation. In a selective medium, on the other hand, typhoid bacilli tend to develop before *Bact. coli*; thus Waldhecker (1935) notes that plating from a tetrathionate brilliant green bile selective medium after 5 hours' and 10 hours' incubation, instead of after 20 hours' only, considerably increased the percentage of positive results (see also Boecker 1935).

It must, in particular, be emphasized that the isolation of typhoid or paratyphoid bacilli from a specimen of fæces depends more on the relative numbers of these bacteria in the particular specimen examined than on any detail of technique. No reliance can be placed on a single negative result, whatever method of examination is employed; and in carriers or convalescents, where the excretion of these organisms may be intermittent, many specimens may have to be examined before a positive result is obtained.

The Agglutination Reaction in Diagnosis.—In applying the agglutination reaction in the diagnosis of enteric infections we must first decide which of the possible causative organisms we shall include in our test. This obviously depends on the actual frequency of the different types of infection in the particular part of the world in which we happen to be working. In Great Britain, for instance, the very great majority of enteric infections are, at the present time, caused by infection with either *Salm. typhi* or with *Salm. paratyphi B*, whereas in British Guiana, *Salm. typhi* and *Salm. paratyphi C* constitute the dominant species (see Giglioli 1930). This problem of the relative frequency of different types of infection also arises in connection with the interpretation of agglutination tests, and will be considered more fully under that head. For the moment we may suppose that we desire to include in our test all the species that usually cause a continued fever of the enteric type, but to exclude those that usually give rise to an acute gastro-enteritis.

This leaves us with *Salm. typhi*, *Salm. paratyphi A*, *Salm. paratyphi B*, *Salm. paratyphi C*, and *Salm. choleræ-suis* var. *kunzendorf*. All these species contain both flagellar and somatic antigens (see Chapter 30), and many observations that have been recorded during recent years have shown quite clearly that the agglutinins acting on both these antigens must be considered.

It has been shown, for instance, that some cases of enteric infection fail to develop H (flagellar) agglutinins while developing O (somatic) agglutinins to relatively high titre (Felix 1924*a*, *b*, 1930, Stuart and Krikorian 1928, Felix and Olitzki 1928, Pijper 1930, Gardner *et al.* 1930, Horgan 1932, Flis and Rosenberg 1934, Bole 1935). A test carried out with formolized broth cultures alone, and thus revealing only H agglutinins, would miss a varying proportion of positive results.

It would appear that the proportion of cases showing O agglutinins only is much higher in some localities than in others. Thus Pijper in South Africa (see Felix and Olitzki 1928) noted 34 persistently negative reactions in 120 cases of undoubted typhoid fever, when

testing for H agglutinins only. Some cases of this type, at least, are infected with O variants of the typhoid bacillus (Olitzki 1928, Gardner *et al.* 1930).

It is equally true that particular samples of serum obtained from typhoid or paratyphoid cases may show flagellar but not somatic agglutinins. Smith (1932) records that 6 of 28 cases of typhoid fever showed H but not O agglutinins when the cases were first examined, while 11 of 42 cases of paratyphoid B fever gave a similar result (see also Downie and Fairbrother 1934, Soman 1934, Flis and Rosenberg 1934, Bole 1935). According to Scott (1941), H agglutinins are seldom absent from the blood of paratyphoid fever cases, though in typhoid fever they may completely fail to appear, or develop late or feebly.

Whether a case ever passes through all its stages developing H but no O agglutinins is as yet uncertain. In any event the lesson is clear—both H and O agglutinins should be searched for in each specimen of blood examined. It must be remembered, however, that occasional cases fail to develop either H, O or Vi agglutinins (Report 1941, 1942*a*).

In selecting suspensions for the serum diagnosis of enteric fever, reference may be made to the Kauffmann-White schema shown on p. 713. There it will be seen that *Salm. typhi* and *Salm. paratyphi A* and *B* all share the common XII somatic antigen. Because of this, and perhaps of other common antigens present in smaller quantity and not represented in the differential diagnostic schema, O agglutinins for paratyphoid bacilli are often demonstrable in typhoid fever, and O agglutinins for typhoid bacilli in paratyphoid fever (Felix 1924*a*, Smith 1932, Cruickshank 1939). It is true that, in persons who have neither been inoculated with T.A.B. vaccine nor suffered previously from any *Salmonella* infection, the O titre for the infecting organism tends to be higher than for the heterologous organism (Gardner and Stubington 1932); but, in general, it is wise to depend for differential purposes on H agglutinins. Even these, as will be seen later, are quite unreliable for distinguishing between the different types of enteric fever, or even for establishing a diagnosis at all, in inoculated persons or in those who have previously been infected with *Salmonella*.

We may note that it is not as a rule necessary to include a Vi suspension, since it is uncommon for Vi antibody to appear in the blood of an active case of enteric fever in the absence of H or O antibody (see Kauffmann 1935), though it may occasionally be useful (Report 1943*a*). The test is said to be of value in prognosis. Bhatnagar (1944), for example, finds that in severe cases of the disease Vi agglutinins are absent, or present in only low titre. On the other hand, cases in which the Vi titre is high—1/50 to 1/250—and is maintained over a number of days, are likely to recover rapidly.

In this country it is usually sufficient in routine practice to test the serum of a suspected patient against the following suspensions: *Salm. typhi* O, *Salm. typhi* H, *Salm. paratyphi B* H, and a composite non-specific *Salmonella* H to detect agglutinins to organisms in the group phase. Rarely it may be necessary to add *Salm. paratyphi C* H, particularly if there is any suspicion of infection with *Salm. choleræ-suis*; though this will usually be detected by the use of the non-specific *Salmonella* suspension. In other countries additional O or H suspensions may be required according to the incidence of various types of enteric fever. To avoid non-specific cross-agglutination the suspensions should be tested before use to ensure their freedom from the so-called X antigen (Topley and Ayrton 1924, Cruickshank 1939). It is always wise to include in a routine test a *Brucella* suspension, since undulant fever may closely simulate typhoid. In cases of con-

tinued pyrexia in which no agglutinins to the organisms already mentioned can be found, the serum should be tested against a suspension of *Leptospira icterohæmorrhagiæ* and of *Proteus OX* 19.

So far we have been considering qualitative results, the mere presence or absence of agglutinins acting on particular antigens; but we actually need to know the concentration in which each of these agglutinins is present in any given specimen of serum.

The results of agglutination tests are commonly expressed as titres, *i.e.*, as the highest dilution of a given serum that will produce agglutination of a given bacterial suspension under specified conditions of time and temperature. We shall, in all cases, wish to determine the real end-point; so that we must specify conditions that will allow this end-point to be obtained. Somatic agglutination, for instance, is slower than flagellar flocculation. Tests with O suspensions should be read after 4 hours' incubation in a water-bath at 50° C., and again overnight. Tests with H suspensions may be read after 2–4 hours under the same conditions. It is usual to employ a geometrical series of dilutions, such as 1 : 20, 1 : 40, 1 : 80, 1 : 160 and so on, and the observed titre is thus subject to a wide margin of error unless some additional precautions are taken. A rough approximation may be made by noting the last tube to show detectable flocculation of the bacilli. By adopting an arbitrary degree of agglutination as a standard, and applying an interpolation table to the actual readings obtained, a much greater degree of accuracy can be attained (see Dreyer and Inman 1917).

But our difficulties do not end here. All suspensions of the same bacterial species are not equally sensitive to agglutination; and if we tested a single sample of serum against a dozen suspensions of *Salm. typhi* prepared from different strains in different ways we might get widely divergent answers. If there were no gross antigenic variations in our strains there would be a limit to such divergence—we should not often find that the titre given by any one suspension was more than, say, two or four times that given by another; but this is a wide margin of technical error.

A valuable lead was given by Dreyer and his colleagues during the first world war (Dreyer, Walker and Gibson 1915, Dreyer and Walker 1916, Dreyer and Inman 1917); and the Standards Laboratory instituted in the Department of Pathology at Oxford under the auspices of the Medical Research Council has since performed an important service in preparing and issuing standard agglutinable suspensions, not only of the flagellated bacilli of the enteric group, but of non-flagellated variants of the same species, and of various other bacteria that are commonly employed in agglutination tests (see Gardner 1920, 1921, 1929). A sensitive suspension of a particular bacterium is adopted as a standard, and any subsequent suspension is compared with this by duplicate testing against an agglutinating serum. The new suspension is then labelled with a number that allows the titre observed with any given serum to be reduced to the titre that would have been observed had the "standard" suspension been employed. Even the use of standard agglutinable suspensions does not ensure uniformity of result. In an international experiment described by Gardner (1937), in which 64 human sera from cases of typhoid or paratyphoid fever were titrated against standard agglutinable suspensions, a surprisingly big variation was recorded between the results of the four different laboratories that took part. Owing to technical errors and differences of one sort and another, and to the bias of individual workers in reading agglutination end-points, it is frankly impossible to expect different laboratories to obtain identical results. This conclusion must always be borne in mind when interpreting titres obtained by other laboratories,

and especially when an attempt is made to over-simplify the problem of serological diagnosis by the establishment of absolute standards.

This brings us to the question as to whether any particular titre, and if so what, can be regarded as a reliable indication of infection with one or other of this group of organisms. The answer is quite definite. *No arbitrary titre can be selected at, or above, which an agglutination can be regarded as "positive" in the diagnostic sense, and below which it can be regarded as "negative". An observed titre for any one of these organisms constitutes one item of evidence which must be considered in relation to all the other evidence available.*

Among the more important ancillary data on which the interpretation of a single agglutination reaction depends are the following:—

(1) The natural level of agglutinins among a random sample of the population in the particular area in which the test is being made.

(2) The stage in the disease at which the sample of blood has been taken.

(3) The state of the patient in regard to previous inoculation with typhoid, or T.A.B., or T.A.B.C. vaccine.

We may discuss these various factors in sequence; but we should note that the average level of agglutinins in a given population, as well as their titre in any particular person, may sometimes be affected by the results of prophylactic immunization.

We shall discuss the available evidence under (1) in some detail, since it has an important bearing on the epidemiology of the disease, as well as on diagnosis. It will be convenient to deal separately with H and O agglutinins and to start with the former.

(1) **Frequency Distribution of Agglutinins in the Population.**—A valuable record of the London population in 1921 is given by Rosher and Fielden (1922), who examined 1,000 samples of serum sent to the laboratory for a Wassermann test, *i.e.*, from patients in whom there was no suspicion of an existing typhoid infection.

These sera were tested against formolized suspensions of *Salm. typhi*, *Salm. paratyphi A*, *Salm. paratyphi B* and certain other members of the *Salmonella* group at dilutions ranging from 1 : 20 to 1 : 640 or more. Since our present knowledge with regard to the diphasic variation of the flagellar antigens was not then available it is impossible to be certain which type of agglutinin was concerned in the flocculation of the diphasic species. Taking the results for the three more important species, with this reservation as regards *Salm. paratyphi B*, we obtain the figures set out in Table 126.

TABLE 126

LONDON

Bacterial Suspension.	Percentage of Sera Agglutinating at 1 : 20 or over.
Salm. typhi	29
Salm. paratyphi A	17
Salm. paratyphi B	23

In 330 instances it was possible to obtain a reliable history as to whether the person had or had not been inoculated. The figures for this sub-sample, again taking the titres of 1 : 20 or over, are set out in Table 127.

TABLE 127

LONDON

Bacterial Suspension.	Inoculated.		Uninoculated.	
	No. of Sera.	Per cent. +	No. of Sera.	Per cent. +
Salm. typhi	149	89	181	3
Salm. paratyphi A	149	58	181	0
Salm. paratyphi B	149	71	181	4

Since many more males than females were inoculated during the war it was to be expected that the figures for the two sexes would differ widely, and this was found to be the case. Thus, among 276 unselected male sera 42 per cent. agglutinated *Salm. typhi* at a titre of 1 : 20; 28 per cent. *Salm. paratyphi A*; and 39 per cent. *Salm. paratyphi B*. Among 165 female sera, 5 per cent. agglutinated *Salm. typhi*; 2 per cent. agglutinated *Salm. paratyphi A*; and 7 per cent. agglutinated *Salm. paratyphi B*.

It is clear that the relatively high proportion of agglutinating sera among the London population as a whole was, in 1921, determined by the presence of a large number of recently inoculated persons.

A similar study was carried out in Manchester a few years later by Smith, Macvie and Newbold (1930); 302 specimens of sera sent to the laboratory for Wassermann tests during the years 1925 and 1926 were tested against formolized suspensions of various bacteria of the *Salmonella* group. In this case type suspensions of the diphasic species were employed, together with a single group suspension to detect the group agglutinins, so that a complete picture of the flagellar agglutinins was obtained over the range of types examined.

Taking first the Manchester population as a whole—and including the results with the type phase of *Salm. paratyphi C*, and the group suspension—the percentages giving agglutination at 1 : 20 or over are set out in Table 128.

TABLE 128

MANCHESTER

Organism.	Percentage of Sera Agglutinating.
Salm. typhi	14·9
Salm. paratyphi A	5·9
Salm. paratyphi B (type)	5·9
Salm. paratyphi C (type)	0·3
"Group" Suspension	6·3

Comparing these figures with those of Rosher and Fielden they show a drop to about a half in the case of *Salm. typhi*, to about a third in the case of *Salm. paratyphi A* and to about a quarter in the case of *Salm. paratyphi B*. Agglutinins for the type phase of *Salm. paratyphi C* appear to be very uncommon in the normal English population. No information was available with regard to the state of inoculation of the persons from whom these blood samples were obtained; but a division into males and females (see Table 129) affords highly suggestive evidence.

TABLE 129

MANCHESTER

Organism.	Percentage of Sera Agglutinating.	
	Males.	Females.
Salm. typhi	23·3	4·7
Salm. paratyphi A	11·6	0
Salm. paratyphi B (type)	9·7	2·0
Salm. paratyphi C (type)	0·0	0·6
"Group" Suspension	7·0	4·7

It will be noted that the figures of Smith, Macvie and Newbold for females agree fairly closely with those of Rosher and Fielden for uninoculated persons of both sexes, or for unselected female sera, so far as *Salm. typhi*, *Salm. paratyphi A* and *Salm. paratyphi B* are concerned. In the case of the latter organism Rosher and Fielden's figure of 4·0 per cent. must be compared with both the paratyphoid B and group figures of Smith, Macvie and Newbold.

The fall in the frequency of flagellar agglutinins among the population as a whole is almost certainly due to a wearing-off of the effect of inoculation. Thus, in the case of *Salm. typhi* Rosher and Fielden found 42 per cent. of agglutinating sera among adult males, compared to the 23·3 per cent. noted by Smith, Macvie and Newbold some six years later.

When we consider titres, as well as the mere presence or absence of agglutinins, the figures provided by Smith, Macvie and Newbold for females (see Table 130) afford a useful guide to the titres we may expect to find in uninoculated adults. The figures under each titre give percentage of sera reacting at that titre, or over. Titres of over 1 : 160 are clearly very exceptional. Below this level there are considerable differences in the frequency of flagellar agglutinins for the different organisms. These conclusions are borne out by the observations of Schwabacher, Ross and Carruthers (1943) made in London and Cambridge about 10 years later.

TABLE 130

MANCHESTER (Females)

Organism.	Percentage of Sera Agglutinating at, or above					
	1:20	1:40	1:80	1:160	1:320	1:640
Salm. typhi	4·7	2·7	0·7	0·7	0	0
Salm. paratyphi A	0	0	0	0	0	0
Salm. paratyphi B (type)	2·0	1·3	0	0	0	0
Salm. paratyphi C (type)	0·6	0·6	0·6	0	0	0
"Group" Suspension	4·7	2·0	1·4	1·4	0·7	0

An extremely interesting contrast to these English figures is afforded by those recorded by Giglioli (1933*a*) for a population of native labourers in British Guiana. Table 131 has been constructed from his data for 350 uninoculated persons, taking the figures for males and females together.

TABLE 131

BRITISH GUIANA (Males and Females)

Organism.	Percentage of Sera Agglutinating at, or above					
	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640
Salm. typhi	24·6	16·0	8·3	4·0	1·7	0
Salm. paratyphi A	9·4	6·9	3·7	1·7	0	0
Salm. paratyphi B (type)	6·0	3·1	2·3	1·1	0	0
Salm. paratyphi C (type)	14·3	11·4	7·1	6·0	2·6	0·6
"Group" Suspension	29·4	20·0	12·9	8·9	2·6	0·9

The figures for H agglutinins acting on *Salm. typhi*, *Salm. paratyphi A*, *Salm. paratyphi C* or the "Group" suspension are all much higher than the corresponding figures for London or Manchester. The discrepancy is greatest in the case of *Salm. paratyphi C*, and this clearly arises from the fact that this type of infection is, as Giglioli (1930) has shown, endemic in British Guiana.

As another contrast we may take the figures given by Alves (1936) for H agglutinins acting on *Salm. typhi*, *Salm. paratyphi A* and *Salm. paratyphi B* among 530 sera from uninoculated natives in Southern Rhodesia. The lowest titre recorded in this series was 1 : 50. At this titre 5·1 per cent. of the sera agglutinated *Salm. typhi*; at 1 : 125 the percentage was 3·8, at 1 : 250 it was 2·2, and at 1 : 500 it was 1·5. No serum agglutinated *Salm. paratyhi A* or *Salm. paratyphi B* in the dilutions tested. Lewin (1934), working in South Africa, records the titres for typhoid H agglutinins among 442 sera from probably uninoculated persons. Of these sera 10·6 per cent. gave agglutination at 1 : 25, 2·9 per cent. at 1 : 100, 1·1 per cent. at 1 : 200, and 0·4 per cent. at 1 : 400—figures which are in near agreement with those of Alves over a similar range.

When we turn to O agglutinins we find our evidence rather less detailed.

So far as figures for this country are concerned, we have combined those recorded by Gardner and Stubington (1932) on 50 normal uninoculated persons in Oxford with those of Beattie and Elliot (1937) on 47 uninoculated students in Edinburgh (Table 132).

TABLE 132

OXFORD AND EDINBURGH. UNINOCULATED PERSONS.

Organism.	No. of Sera Examined.	Percentage Agglutinating at, or above		
		1 : 20 or 1 : 25.	1 : 40 or 1 : 50.	1 : 80 or 1 : 100.
Salm. typhi O	97	26	5	1
Salm. paratyphi A O	50	0	0	0
*Salm. paratyphi B** O	97	22	10	5†

* The suspension employed was prepared from *Salm. typhi-murium*, which normally has the same O antigens as *Salm. paratyphi B*.

† One person had recently suffered from food poisoning.

Very similar results were reported by Schwabacher, Ross and Carruthers (1943), who examined a total of 560 "normal" sera. The end-points varied with the length of incubation and the method of reading, but with one or two exceptions the highest O titres met with against both *Salm. typhi* and *Salm. typhi-murium* were 1 : 80.

TABLE 133

BRITISH GUIANA

Organism.	Percentage of Sera Agglutinating at, or above					
	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640
Salm. typhi O.	16·3	3·7	0·9	0·3	0·3	0
Salm. paratyphi B O. . . .	20·3	5·7	2·0	0·3	0·3	0
Salm. paratyphi C O. . . .	19·4	9·4	3·1	2·0	0·9	0·3

Giglioli (1933*a*) included O agglutinins in his survey among the native labourers in British Guiana (Table 133). It will be noted that the O agglutinins among this sample of the population show no excess, where comparison is possible, over the English figures, in striking contrast to the marked excess shown with H agglutinins.

Horgan (1932) has tested 70 sera from Sudanese with no evidence of enteric infection, and no history of inoculation. At a titre of 1 : 25, 7·1 per cent. agglutinated an O suspension of *Salm. typhi*, 4·3 per cent. agglutinated an O suspension of *Salm. paratyphi B*, while no serum agglutinated an O suspension of *Salm. paratyphi A*.

Lewin (1934) gives the following percentages for typhoid O agglutination at various titres among the 442 sera examined by him: 45·0 per cent. agglutinated at 1 : 25, 4·5 per cent. at 1 : 100, 2·0 per cent. at 1 : 200, and 1·8 per cent. at 1 : 400.

Alves (1936) gives figures for typhoid O agglutinins among 300 sera from normal uninoculated natives in Southern Rhodesia: 9·33 per cent. gave agglutination at 1 : 50, 2·67 per cent. at 1 : 100, 2 per cent. at 1 : 250 and 1 per cent. at 1 : 500.

Where comparison is possible these figures are not widely discrepant; and they suggest that the frequency and concentration of O agglutinins in different parts of the world differ much less than the frequency and concentration of H agglutinins. This is not surprising in view of the fact that the antibody response to specific stimulation with the O antigens is known to be more transitory than the response to the H antigens. There are some reasons for believing that the normal O agglutinins, when present to low titre only, represent normal antibodies of the type that have not arisen in response to infection (see Chapter 49).

Further surveys of this type are badly needed. We should have an accurate knowledge of the level of H and O agglutinins among the normal population in all those parts of the world in which the laboratory diagnosis of enteric infections is undertaken. Moreover, these surveys should be carried out by a standardized technique, and with standard reagents. The figures quoted above are not, indeed, strictly comparable with one another because these requirements were not always fulfilled.

(2) Stage of the Disease.—The next factor that we have to consider in a diagnostic agglutination test is the stage of the disease at which the sample of blood was taken.

Reference to Fig. 283, p. 1520 and to Figs. 260, 261, p. 1121, will indicate that the significance of a positive or negative agglutination test, or of a positive result to any given titre, varies according to the stage of the disease at which the sample of blood was taken.

It is clear that little importance can be attached to a negative agglutination test on a sample of blood taken during the first week of illness. It does *not* follow that a test made at this time is useless. It is often of the greatest value since, taken in conjunction with tests made at a later period, it often enables us to demonstrate that a significant rise in agglutinins has occurred; the demonstration of such a rise has far more diagnostic significance than the demonstration that any particular sample of serum gives agglutination to a particular titre, unless that titre is a very high one.

As an illustrative example, bearing in mind our evidence with regard to the distribution of normal agglutinins, let us suppose that we find that a sample of serum, taken from a patient in the first week of the disease, gives an H titre of 1 : 20 against the typhoid bacillus. If the sample had been taken in British Guiana, this finding would have almost no significance, since about 1 normal person in 5 would give this titre. If it had been taken in South Africa, it would have been more suggestive. If it had been taken in England it would have been still more suggestive; only about 1 normal person in 20 would give this titre. If we found, in addition, an O titre of 1 : 50, the significance of the H titre of 1 : 20 would be greatly increased. Similarly, a 1 : 20 H titre against *Salm. paratyphi C* in England would be very suggestive of infection. In British Guiana it would have almost no significance.

If, however, we found these titres in a specimen of serum taken from a patient towards the end of the second week of the disease, or in the beginning of the third, their significance would be entirely altered. By that time a patient has usually developed an H titre well above 1 : 20, and an O titre well above 1 : 50. If we found such low titres at so late a period we should be inclined to suspect that they were due to some cause other than active infection.

Clearly we should like to have figures for the average H and O titres against the causative organism at each period of infection, and for each type of enteric infection; but we have to be satisfied with something much less than this, because records that afford all the necessary data are by no means plentiful. Such figures as we have, however, give us a very useful lead.

Gardner and Stubington (1932) in 40 cases of typhoid fever record H titres of which all but 3 were above 1 : 125, 30 were 1 : 250 or over, and 14 were 1 : 1,000 or over. Of the O titres all were over 1 : 125, 37 were over 1 : 250, and 13 were over 1 : 1,000. In 40 cases of enteric fever caused by *Salm. paratyphi B* the level of H agglutinins tended to be rather higher, but the level of O agglutinins, either for *Salm. typhi* or *Salm. paratyphi B*, definitely lower. Twenty-two of the 40 sera showed an O typhoid titre of less than 1 : 125, and 17 showed an anti-paratyphoid B O titre below this limit. Somewhat similar results are recorded in several of the papers referred to above, and in others not quoted.

In most instances it is not possible from the records to determine the day of disease on which the different specimens were taken; but it seems a fair conclusion from the data that the great majority of typhoid infections will, during the 3rd week of the disease, show an H titre well over 1 : 100, and usually over 1 : 200, and an O titre of 1 : 100 or over. With paratyphoid infections the H titre against the causative organism will tend to be at least as high as that observed in typhoid fever, but the O titre, particularly if an O typhoid suspension is employed, may be somewhat lower.

(3) Effect of Previous Inoculation.—Let us now turn to the third factor that may influence the interpretation of our results, the effect of previous inoculation with a typhoid, or T.A.B. vaccine. This factor is particularly important in the

diagnosis of enteric infections among troops in war-time, or in countries where the risk of enteric infection is so high that many of the white or native population are inoculated.

Considerable assistance is gained, in the solution of this technical difficulty, by the difference in the nature of the antibody response to the H and O antigens. Felix (see above), who first emphasized the value of this method, originally stated that O agglutinins were not formed in response to antityphoid inoculation, and that hence O agglutination had the same diagnostic value in the inoculated as in the uninoculated. This, however, is not the case.

Thus Gardner (1929) found that of 6 persons with a history of inoculation in past years 1 gave O agglutination with *Salm. typhi* at a titre of over 1 : 200 and of 11 persons inoculated 4 weeks before testing 3 gave titres of this order. To check these findings a healthy adult, who had been inoculated some 14 years earlier, was given 2 inoculations of T.A.B. vaccine and his agglutinins were determined at frequent intervals during the following month. The flagellar agglutinins were raised from a titre of 1 : 185 to a titre of 1 : 1,500; the O agglutinins were raised from zero to 1 : 50; both thereafter sank.

Mudd (1932) showed that sera collected 10 days after the third injection of a T.A.B. vaccine contained O agglutinins to a significantly higher titre (1 : 80 to 1 : 320) than the average normal uninoculated controls; and Horgan (1932) and Giglioli (1933*b*) record similar results.

As compared with these O titres, however, the H titres developed after typhoid and paratyphoid inoculation are very high. They usually attain a level of 1 : 500 or more, and often well over 1 : 1,000. What is much more important the O agglutinins disappear from the circulation far more rapidly than the H agglutinins.

Smith (1932) examined the sera of 17 normal persons who had been injected with typhoid vaccine between the years 1915 to 1929. All 17 showed flagellar agglutinins for *Salm. typhi* at titres varying from 1 : 50 to 1 : 200; two of them showed O agglutinins, one at a titre of 1 : 50, the other at a titre of 1 : 200. Wyllie (1932) examined the sera of 22 persons who had received prophylactic injections of typhoid vaccine between 1928 and 1931; 13 showed no O agglutinins at 1 : 25, 9 reacted at or above this titre, the highest titre reached being 1 : 100 (see also Giglioli 1933*b* and others).

A valuable series of observations on men of the Royal Air Force, illustrating the high and more persistent titres of H agglutinins and the low and more transitory titres of O agglutinins after inoculation, is recorded by Downie and his colleagues (Report 1942*c*). Many of the men had had several injections of T.A.B. vaccine during the previous three years, but the results were classified according to the time that had elapsed since the last injection (Table 134).

It will be seen that the O agglutinin response to *Salm. typhi* was very slight, never reaching 1 : 200, even in those who had received their last injection within three months of testing; but that the H agglutinin response for all three organisms was much higher, in some instances reaching or exceeding a titre of 1 : 1,600 for the two paratyphoid suspensions one to three years after inoculation. Similar results on inoculated naval ratings have been recorded by Beattie and Elliot (1937). The O titres recorded by these observers are a little higher than those reported by Downie, but this was probably due to a difference in reading of the end-point, Beattie and Elliot apparently using the standard agglutination end-point recommended by the Oxford Standards Laboratory and Downie using the +++ agglutination end-point recommended by Felix. (See also J. F. Wilson 1945.)

TABLE 134

PERCENTAGE DISTRIBUTION OF END TITRES AGAINST *Salm. typhi* O AND H AND *Salm. paratyphi A* AND *B* H SUSPENSIONS.

Time since Last Inoculation of T.A.B. Vaccine.	No. of Sera Tested.	Suspension	Per cent. of sera giving End Titres of					
			<1:100	1:100 <1:200	1:200 <1:400	1:400 <1:800	1:800 <1:1,600	1:1,600 or over
1 week to 3 months	198	TO	95·0	5·0	—	—	—	—
		TH	30·3	21·7	23·8	16·1	6·6	1·5
		AH	5·0	7·6	15·7	30·3	29·8	11·6
		BH	4·0	10·1	20·2	24·8	27·2	13·6
3 months to 1 year	70	TO	97·1	2·9	—	—	—	—
		TH	37·2	25·7	20·0	12·9	4·3	—
		AH	15·7	21·4	12·9	30·0	14·3	5·7
		BH	10·0	10·0	21·4	28·6	18·6	11·4
1 year to 3 years	29	TO	100·0	—	—	—	—	—
		TH	41·4	31·0	17·8	3·8	10·3	—
		AH	34·5	—	20·6	20·6	10·3	13·8
		BH	17·2	20·6	13·8	31·0	10·3	6·9
Total sera 1 week to 3 years	297	TO	96·0	4·0	—	—	—	—
		TH	33·0	23·6	21·9	14·1	6·4	1·0
		AH	10·4	10·1	15·5	29·2	24·2	10·4
		BH	6·7	11·1	19·9	26·2	23·6	12·5

There are, then, good grounds for Felix's contention that O agglutinins are of more significance than H agglutinins in the diagnosis of enteric fever in inoculated persons.

There is another way in which this problem has been attacked. By taking several samples of blood from the same patient at different stages of the disease we may construct an "agglutinin curve" and base our diagnosis on fluctuations in titre rather than on the titre obtained with any one sample (see Dreyer, Walker and Gibson 1915, Dreyer and Walker 1916, Dreyer and Inman 1917, Perry 1918, Topley, Platts and Imrie 1920). When an inoculated person becomes infected, the response to infection is superimposed on the residual effect of the earlier response to inoculation, and we get the usual "infection" curve, starting not from zero, but from a base line representing the residual "inoculation" agglutinins. Since the rise in titre is more rapid than the fall, the most useful information will be obtained by repeated tests carried out during the first 3 weeks of infection; but the early part of the fall from the maximal titre attained is usually steep enough to show a significant drop in titre during the early weeks of convalescence.

Experience of this method has shown that, though relatively large fluctuations in the titre of O agglutinins are very suggestive, the same diagnostic significance cannot be attributed to changes in the titre of H agglutinins. Many years ago, Felix (1924*b*) reached the conclusion that H agglutinins were so liable to non-specific stimulation in inoculated persons who contracted a febrile infection that they could not be relied upon to indicate the presence of a specific infection with the typhoid or paratyphoid bacillus. Though it is clear from the observations of Perry (1918) and Topley, Platts and Imrie (1920) that inoculated persons may suffer from febrile infections of many kinds without showing any detectable fluctuations in the titre of flagellar agglutinins, experience has shown that in some persons

a very considerable rise in the titre of these agglutinins may occur during the course of non-enteric pyrexial infection, and that little weight can therefore be attached to such a rise in reaching a diagnosis of the disease.

Summarizing, we may repeat once more that no arbitrary titre of H or O agglutinins can be accepted as diagnostic of active infection. Subject, however, to the conditions and limitations discussed in the previous few pages, we may say that in this country a titre of 1/50 for H agglutinins or of 1/100 for O agglutinins, observed during the first ten days of the disease in a patient who has no history of inoculation or of previous enteric infection, may be regarded as strong presumptive evidence of infection with organisms of the typhoid or paratyphoid group.

In *inoculated* persons or in persons with a previous history of enteric infection, no attention can be paid to H agglutinins; even a considerable rise in titre is of little or no diagnostic importance, since this may be due to non-specific causes. On the other hand, O agglutinins, if present in a titre of 1/100 or over, six months or more after inoculation, are strongly suggestive of active infection, particularly if on re-examination they are found to have altered in titre. Many cases, however, of enteric fever may run their course without ever reaching or exceeding such a titre, thus rendering it impossible to arrive at a diagnosis by serological methods alone. In the rare cases in which both H and O agglutinins are absent from the blood throughout the disease, the Vi agglutination test may prove helpful (see Report 1943*a*).

The Epidemiology of Enteric Fever.

It would take us altogether beyond the scope of this book to discuss in any detail the extensive literature bearing on the epidemiology of enteric fever. It will, however, be useful to summarize the conclusions to be drawn from the available evidence, quoting certain illustrative records and reports, many of which must, of necessity, be arbitrarily selected from the numerous examples available.

The main conclusions which emerge quite clearly from the mass of evidence on record are that the single important source of enteric infection consists in typhoid or paratyphoid bacilli which are living, or proliferating, within the bodies of infected persons; and that the whole problem of prevention, neglecting for the moment the possibility of active immunization, consists in stopping the various routes by which the bacilli may pass from the intestine of one individual to the mouth of another.

Moreover, the evidence obtained during surveys of H agglutinins among the normal population in different parts of the world, where different types of enteric infection are endemic, shows quite clearly that in this, as in other diseases, the frequency of infection is far greater than the frequency of the clinical disease, so that infection from carriers and ambulant cases is almost certainly more important in its total effect than infection from clinically recognized cases (see Chapter 56 and discussion below).

Beyond this point the epidemiology of enteric fever cannot profitably be considered as a whole. Though the original source of infection is the human intestinal or urinary tract, the route of epidemic spread differs notably in typhoid and in paratyphoid fever. The evidence in favour of this conclusion has been carefully marshalled by Savage (1942), whose study affords an admirable example of the inductive method of arriving at broad generalizations by the analysis of numerous

diverse observations. Speaking broadly, it may be said that typhoid fever can probably be caused by the ingestion of relatively small numbers of bacilli, whereas paratyphoid fever does not usually result unless large numbers of bacilli are ingested. It is probably for this reason that, though both diseases may be carried by food, typhoid fever is often borne by water and shell fish, whereas these vehicles are rare in the genesis of paratyphoid outbreaks.

The part played by *water* in the spread of typhoid fever was first established by the painstaking observations of William Budd (1856, 1873) in this country, and has been confirmed by experience in many parts of the globe.

Several classical examples of water-borne outbreaks are on record, such as those at Caterham in 1879, at Worthing in 1893, at Maidstone in 1897, at Lincoln in 1905, at Bolton-upon-Dearne in 1921, and at Malton in 1932 (see Greenwood 1935, Forbes 1938). More recently the Croydon outbreak of 1937 may be mentioned, in which there was a total of 310 cases with 43 deaths (Report 1937; see also Holden 1939). In the United States Gorman and Wolman (1939) were able to collect records of 399 outbreaks of typhoid fever between 1920 and 1936, comprising a total of 12,585 cases, of which 862 proved fatal. The corresponding figures for Canada during the same period were 71 outbreaks, 3,437 cases, and 192 deaths.

Among the most frequent causes of contamination of water are cross-connexion of a main with a polluted water supply, seepage of surface water or sewage into a gravity conduit, surface contamination of shallow wells, and inadequate filtration or chlorination (Gorman and Wolman 1939). It may be noted that the evidence inculpating a particular water supply is almost always circumstantial; partly because the isolation of typhoid bacilli from water is beset with technical difficulties, and partly because the effective pollution is often temporary or intermittent, and has usually ceased by the time the water supply falls under suspicion. The evidence is, none the less, convincing enough. Water-borne outbreaks are generally characterized by a typically explosive onset; the curve of notifications rises suddenly and steeply, the majority of the cases developing within a relatively few days. The curve for the whole epidemic is often characteristically skew, the primary cases due to direct infection from the water supply being followed by secondary crops of contact cases. Less often, particularly when infection of the water is slight and intermittent, instead of an explosive outburst, there is a series of scattered or "dropping" cases or small groups of cases occurring over a considerable period of time, and affecting only a small proportion of the consumers. Suspicion of the water should always arise if building operations have been proceeding on a gathering ground, or if extensions, repairs or other alterations have been going on in the supply services. The infrequency of water-borne outbreaks of paratyphoid fever has already been noted. One small but clear-cut outbreak in the little village of Brixworth in Northamptonshire, in which a shallow well water was found to be heavily contaminated with paratyphoid B bacilli, was described by Jones, Gell and Knox (1942); and Savage (1942) gives references to two or three other outbreaks suspected of being water-borne. Franklin and Halliday (1937) mention one small outbreak in Maryland of paratyphoid A fever in which a common well was exposed to infection by a person convalescent from the disease. In view of the known survival of paratyphoid bacilli in sewage and the not infrequent access that these organisms must gain to water supplies, their failure, in contrast to typhoid bacilli, to set up disease is most striking.

Shell fish, particularly oysters, are another important source of infection in

typhoid fever, though rarely incriminated in paratyphoid fever. They are often bred or fattened in the sewage-polluted waters of tidal estuaries, and they are usually consumed uncooked. Numerous epidemics are on record which can be traced to this source (see Gay 1918), and sporadic cases are likewise common. In France alone Belin (1934) estimated that during the previous fifteen years more than 100,000 cases of typhoid fever had occurred due to the consumption of shell fish, of which 25,000 had ended fatally. The relation between the consumption of shell fish and the incidence of typhoid fever in a large city has been considered in some detail by Niven (1910) in a study of the seasonal prevalence of typhoid fever in Manchester.

Milk follows close on water as an important source of sudden and massive herd infection. Ballard, in pre-bacteriological days, adduced good circumstantial evidence for inculpating a polluted milk supply in a localized epidemic at Islington. Schüder (1901), who studied the records of 640 epidemics, attributed 110 to contaminated milk.

In this country there are numerous records of milk-borne outbreaks of typhoid and paratyphoid fever, of which the 1931 outbreak of paratyphoid fever at Epping affecting 260 persons and causing 8 deaths (Bullough 1931), and the 1936 outbreak of typhoid fever at Bournemouth, Poole and Christchurch affecting at least 718 persons and causing about 70 deaths (Shaw 1937), are among the more important of recent years. In the United States Borman, West and Mickle (1935) were able to collect records of 574 milk-borne outbreaks of typhoid fever during the years 1910 to 1933, comprising 8,459 cases and 406 deaths. In Canada between the years 1912 to 1937 there were 47 milk-borne outbreaks of typhoid fever, affecting 6,701 persons and causing 692 deaths (Defries 1938); of these, the most serious was the Montreal outbreak of 1927, which caused, in the two waves that occurred between the middle of March and the beginning of July, no fewer than 4,849 cases and 489 deaths (Report 1927, Boucher 1927).

The organisms may gain access to the milk from the imperfectly cleansed fingers of attendants in the milking shed or dairy, or from contamination of milking utensils with infected water. Since both typhoid and paratyphoid bacilli can multiply in milk at ordinary atmospheric temperatures, even an initial contamination may prove dangerous. Though milk-borne outbreaks, like those due to water, are often characteristically explosive in their onset, scattered or "dropping" cases may be caused by milk, when the infection is confined to one or two churns or bottles or is intermittent.

Apart from liquid milk, *other dairy products* or substitutes such as cream, artificial cream, synthetic cream, custard and ice-cream, are frequent vehicles for the enteric group of organisms, particularly paratyphoid bacilli. Of the 40 paratyphoid outbreaks analysed by Savage (1942), 8 were due to liquid milk, 16 to cream and cream cakes, 2 to ice-cream, 1 to trifle, 1 to cream cheese, and 2 to other foods. Several outbreaks of typhoid fever in Canada have been ascribed to the consumption of unripened hard cheese made from raw milk handled by chronic carriers (Bowman 1942, Gauthier and Foley 1943, Menzies 1944). Most of these products are prepared in the home, a dairy, or a bakery, and become contaminated from human sources. One outbreak in Liverpool was traced to loaves of bread handled by a paratyphoid carrier (Frazer, Glover and Glass 1937). On the whole, the evidence suggests that ambulant cases or transitory carriers are most often responsible for contamination of food with paratyphoid bacilli, and chronic carriers for contamination with typhoid bacilli. Chronic carriers of

paratyphoid bacilli and ambulant cases of typhoid fever are said to be relatively uncommon (Scott 1941).

In outbreaks both of typhoid and of paratyphoid fever the typical symptoms of the disease may be preceded by acute gastro-enteritis coming on shortly after consumption of the infected water or food (see Savage and White 1925, Robertson 1936, Warren 1941). These early symptoms may pass imperceptibly during the following week or so into those characteristic of enteric fever, or there may be a remission before the onset of enteric symptoms, or there may be complete and lasting recovery.

Thus, in the Consett paratyphoid outbreak in 1940 which was due to home-made trifle infected from a profuse faecal and urinary excreter who was probably in the convalescent stage of an ambulant infection, of 56 patients admitted to hospital, 7 contracted enteric fever after an incubation period of six to twelve days; 17 suffered from gastro-enteritis coming on 12–72 hours after consumption of the trifle and gradually passed into the enteric state; 17 suffered from gastro-enteritis, recovered more or less completely, and then developed enteric fever after an interval of a few days; 9 suffered from gastro-enteritis and recovered completely; and 6 suffered from no symptoms at all but were proved bacteriologically to be infected.

Whether the initial gastro-enteritis represents the first reaction of the tissues to the specific infecting organisms, or whether it is due to other organisms capable of causing food poisoning, or to pre-formed toxins, is still in doubt.

Human hands are probably the principal agents in the conveyance of typhoid or paratyphoid bacilli from fæces to food; but there is another agent, the fly, which may be of considerable importance as a carrier when allowed access to infected excreta. Its activities in this direction are particularly dangerous in tropical countries, and under the imperfect sanitary conditions which often prevail among armies on active service. Reed, Vaughan and Shakespeare (1899) drew attention to the clear association between the prevalence of typhoid fever among the American troops in the Spanish-American War, and the exposure of excreta; and suggested that flies played an important part in the spread of the disease.

The mechanism involved in the carriage of *Salm. typhi* by flies has been studied experimentally by Firth and Horrocks (1902), Ficker (1903) and Graham-Smith (1910). The danger of contamination from the soiled feet, or proboscis, appears to be of relatively short duration; but the observations of Ficker and of Graham-Smith suggest that a far more important source of infection is provided by the fly which itself becomes infected, and carries the bacilli for some days in its intestinal canal. The observations of Faichnie (1909), who examined flies caught in infected areas, and isolated typhoid bacilli on several occasions from the crushed bodies of the insects after the exterior had been sterilized by flaming, point in the same direction (see also de la Paz 1939).

The Typhoid and Paratyphoid Carrier.—As has been indicated above, the ultimate source of enteric infection would appear, in all cases, to be the excreta of an infected human being, and this source is seldom remote. There is little evidence that typhoid or paratyphoid bacilli survive for any length of time outside the human body under natural conditions. The mode of survival of the bacilli throughout non-epidemic times, and the starting-point of outbreaks in localities which had long been free from the disease, remained in doubt until the early years of the present century, when the problem was solved during the intensive investigation undertaken in S.W. Germany under the auspices of Robert Koch. Horton-Smith

(1900) had previously recorded the case of a urinary carrier of *Salm. typhi*, but the full significance of the chronic carrier was not appreciated until the results of the German campaign were reported. (For an excellent description and discussion of these results see Ledingham and Arkwright 1912.) Koch's thesis, that the convalescent carrier would probably be found to be the main source of the persistence of infection within a community, was soon established on a firm basis by the findings of the Commission (Frosch 1903, von Drigalski 1904); and von Drigalski established the fact that the typhoid bacillus might lead a prolonged existence within the human body, and be excreted in the fæces.

Before quoting records of frequency, it is necessary to define our terms. In the older literature there is some confusion owing to the use of the same term in a different sense by different authors; but there is now fairly general agreement on the use of the word "temporary" or "transient" for carriers who excrete the bacillus for not more than a year, and the word "chronic" for carriers of over a year's standing. Temporary carriers are sometimes divided into "passive" carriers who excrete the organism without suffering from clinical symptoms, and "active" carriers who are suffering or convalescing from a clinically recognizable attack of the disease (see Scott 1941); but we can see little practical value in this distinction and we shall not employ these terms ourselves. There is some serological evidence to suggest that persons may harbour the infecting bacilli in the tissues for a long time without excreting them in the urine or fæces (see Pijper and Crocker 1943). Though the term "carrier" is strictly applicable to such persons, it would in practice be misleading to use it in this sense without some qualification, such as "closed." Excretion, even though only intermittent, is always regarded as part and parcel of the carrier state. It would, of course, be difficult to prove that excretion never occurred; and it is wiser to regard carriers of this type as potential excreters.

In considering the frequency of carriers we must realize that our technique of demonstrating typhoid and paratyphoid bacilli, especially in the fæces, is far from perfect. With the great technical improvements that have resulted in recent years from the introduction of more selective media, the idea has been growing that intermittency of excretion is less frequent than used to be thought, particularly in paratyphoid carriers (see Holt and Wright 1942). Though the methods now in use permit the detection of small numbers of organisms in the fæces more readily than before, it may be doubted whether their application will lead to any substantial modification of our estimate of the proportion of permanent carriers of typhoid bacilli. Many of these excrete the organisms in large numbers; and of the remainder many can be detected by the older media provided repeated examinations are made. Our knowledge, however, is still very deficient, and all our conclusions must be expressed with caution. We may deal first with the *typhoid carrier*.

Lentz (1905) found that 4·5 per cent. of 400 typhoid convalescents excreted *Salm. typhi* for more than 10 weeks, and 3 per cent. for longer than 13 months. Brückner (1910) found 12 carriers among 316 persons who had suffered from typhoid fever in previous years. Kayser (1907) carried out a re-examination of specimens of urine and fæces from 101 persons who had passed through an attack of typhoid fever at least one year earlier, and had been discharged as free from bacilli; 3 of these were found to be again excreting the infecting organism. Gill (1927) refers to the finding in Alabama of 9·5 per cent. of chronic typhoid carriers among a group of 348 persons who had suffered from typhoid

fever or from continued fever of several weeks' duration more than a year previously. Gray (1938) in Mississippi found that of 244 proved cases of typhoid fever, 8 continued to excrete the bacilli for over a year; it is to be noted that several patients excreted the bacilli for 3 to 9 months after recovery, but ceased to do so after 12 months. Ames and Robins (1943) in New York State refer to 90 chronic carriers resulting from 3,130 typhoid patients notified during the years 1930 to 1939.

From these figures it is fairly clear that about 3 per cent. of recognized clinical cases of typhoid fever become permanent carriers, though the proportion may in some places be higher.

Klinger (1909), in summarizing the results obtained to that date, gives records of 431 carriers; 211 of these did not excrete the bacilli for more than three months; 220 excreted them for a longer time. The distribution of these carriers, according to sex, and according to the presence or absence of a history of typhoid fever, was as follows:

	Males.	Females.
Carriers of less than 3 months' duration		
(*a*) History of attack	31	61
(*b*) No history of attack	58	61
Carriers of more than 3 months' duration		
(*a*) History of attack	33	143
(*b*) No history of attack	5	39

Two points of interest emerge from this distribution: (*a*) the high proportion of carriers in the first group in which there was no history of a previous attack, and (*b*) the striking preponderance of females among the second group of carriers. The latter point is of particular importance; and it may be noted that the difference between the sexes in this respect is of the same order as the difference in the occurrence of gall-stones. Klinger's data also show that transitory carriers occur mainly in children and young adults, chronic carriers among those in middle or later life.

Our information on the frequency of typhoid carriers among atypical cases, contacts, non-contacts in epidemic or endemic areas, and the population at large, is somewhat scanty, but it is sufficient to indicate that the distribution is of the general type discussed in Chapter 56.

Billet and others (1910) record the isolation of *Salm. typhi* from a high proportion of clinically atypical cases, during an outbreak of typhoid in an infantry regiment. The bacillus was isolated from the blood in 7 of 39 suspects, and from the fæces in 13 of 64 (20·3 per cent.) Semple and Greig (1908) isolated *Salm. typhi* from the fæces of two nursing orderlies, in typhoid wards, who had never shown any clinical evidence of the disease.

Klinger (1906) examined the excreta of 1,700 persons, living in an area in which typhoid fever was endemic, and isolated *Salm. typhi* in 15 cases. Eleven of these presented no clinical symptoms, either before or after the isolation of the bacilli. In 9 cases the bacilli were found once only. In 2 they were found 3 times; but in no case did they persist longer than 14 days. The other 4 carriers were of the chronic type. Two had passed through an attack of typhoid fever in previous years; from the remaining 2 no history could be obtained. Welch, Dehler and Havens (1925) report the discovery of 39 carriers of *Salm. typhi* during the examination of 1,076 persons engaged in the milk trade, in an area in which enteric fever had been prevalent for years. This carrier rate (3·6 per cent.) is exceptionally high, even for an endemic centre.

Among the population at large the carrier rate is probably very low. Minelli (1906) discovered one carrier among 250 inmates of the town prison of Strassburg—0·4 per cent. Prigge (1909) records 0·29 per cent. of carriers among a sample of 10,841 persons in Saarbrücken. Rosenau, Lumsden and Kastle (1909) found 3 carriers among 993 persons in

Washington. These figures are curiously similar, but the actual variation in the frequency of carriers among the general population is probably wide, and all we can safely conclude is that the average frequency is well under 1 per cent. In many localities it is probably considerably less. In New York State, exclusive of New York City, Ames and Robins (1943) calculated that in 1940 there were about 42 typhoid carriers per 100,000 population.

On the main point at issue the evidence leaves no doubt. Within any community in which enteric fever is endemic, there will be found transient or chronic carriers, who are acting as foci of infection; the isolation of every overt case of illness would not suffice to stamp out the disease.

With regard to contact cases Klinger (1909) records some interesting observations on the period of the disease at which infection is transmitted to the contact. In an investigation of 812 such cases he reached the conclusion that the distribution, in time, of the contact infections was as shown in Table 135.

TABLE 135

Period of Disease.	No. of Contact Cases.	Period of Disease.	No. of Contact Cases.
Incubation period:			
1st week	33	4th week	59
2nd ,,	150	5th ,,	34
Illness:		6th ,,	22
1st week	187	7th ,,	14
2nd ,,	158	8th ,,	16
3rd ,,	116	9th ,,	15
		10th ,,	8

It is obvious that the evidence on which such figures are based must be largely circumstantial. If they are correct it would appear that 183 of 812 contact cases, or more than 20 per cent., were infected during the incubation period of the primary case—at a time when the disease could not have been diagnosed, and when opportunities for its spread would be increased by the fact that the infected person would not be confined to bed. To such primary carriers (*i.e.*, those in the incubation stage of the disease) must be added the relatively numerous ambulatory cases of mild and atypical infection which occur during any extensive epidemic.

Turning now to the *paratyphoid carrier*, we find our evidence less well documented.

Leishman (see Savage 1942) analysed the results obtained at the central enteric depôt at Addington Park during the war of 1914–18. Of 1,425 cases of paratyphoid B fever, 43 (3 per cent.) were found to be still excreting the organisms after six months. At the German military convalescent depôt at Spa, Bumke (1926), who studied 338 fæcal carriers of paratyphoid B bacilli, found that 60–70 per cent. of them ceased to excrete the organism within three months, and 10–20 per cent. within three to six months; 20–30 per cent. were still excreting the bacillus after six months. In an outbreak at Liverpool, Glass and Wright (1937) found that the rate of clearance of paratyphoid bacilli from the fæces was most rapid during the second month; about 4 per cent. of convalescents were still excreting the organisms after 15 weeks. In a later outbreak at Liverpool, described by Holt, Vaughan and Wright (1942), the proportion of patients who continued to excrete paratyphoid bacilli after 16 weeks was 5 per cent. The rate of clearance, however, may be much more rapid. In the Bristol outbreak of 1940, for example, described by Davies, Cooper, Wiseman and Davies (1940) the proportion of carriers had fallen to 6·9 per cent.

after six weeks, as opposed to figures of 67 and 44 per cent. respectively for the two Liverpool outbreaks just quoted. In the Kettering outbreak described by Gell and Knox (1942) 3·6 per cent. of the patients were still excreting paratyphoid B bacilli after seven months.

The proportion of chronic carriers of paratyphoid B bacilli is still a matter of conjecture. Gill (1927) mentions that among 348 persons who gave a history of typhoid fever or of continued fever a year or more previously, 12 carriers of paratyphoid A or B bacilli were found. There is reason to believe that most of these were paratyphoid A carriers, but the exact figures are not given. All we can say at present is that chronic carriers of paratyphoid B bacilli do occur, but that their frequency is unknown.

There is little exact information on the duration of excretion of paratyphoid A bacilli, but the observations of Bumke (1926) suggest that these organisms disappear from the fæces at a considerably greater rate than typhoid bacilli.

In regard to the danger of the chronic carrier, it is only necessary to say that the literature contains numerous and well-authenticated records of their activities. For accounts of such classical instances as the case of the Strassburg Master-Baker's Wife, the case of the Folkestone Milker, or the case of " Typhoid Mary," the student is referred to the excellent monograph of Ledingham and Arkwright (1912), and to a paper by Soper (1939). It need only be noted that in these, as in almost all subsequent cases, the carrier who has achieved publicity has been in some way concerned with the handling of food, and that a large proportion of milk-borne epidemics, or small outbreaks due to the consumption of such food-materials as ice-cream, have been traced to a carrier who has been concerned in their handling or transport.

It may be added that, in searching for a typhoid carrier among a given sample of the population, much help will be obtained by testing the serum of all suspects for agglutinins before proceeding to the cultural examination of fæces and urine. A carrier often shows the presence of H or O agglutinins, even though only in low titre; but these will afford little or no useful information if the person has been inoculated with T.A.B. The observations of Felix (1938*a*) have shown that much greater value attaches to the demonstration of Vi agglutinins. In chronic typhoid carriers the frequency of a positive Vi reaction in the serum appears to be very high. Felix's observations have been confirmed by Eliot (1940), Eliot and Cameron (1941), Klein (1943) and several other workers (see Pijper and Crocker 1943). It is clear, however, that though probably over 90 per cent. of chronic carriers contain Vi agglutinins in their serum, the presence of Vi agglutinins is in no sense diagnostic of the carrier state.

Horgan and Drysdale (1940), for example, in the Sudan found that 46·5 per cent. of sera taken for the Kahn test contained Vi agglutinins in a titre of 1 : 5 or higher. Davis (1940) in Southern Rhodesia obtained a figure of 7·5 per cent. for sera taken from 656 natives at random, but in only one of the 26 positive subjects examined was he able to demonstrate the presence of typhoid bacilli by culture. Eliot and Cameron (1941), searching for carriers in Maryland, found 8 persons among 100 studied with a Vi serum of 1 : 20 or over; from only 4 of these were typhoid bacilli isolated from the fæces. Pijper and Crocker (1943) in South Africa examined the serum of personnel employed, or applying for employment, in dairies, waterworks, or the General Hospital, Pretoria. Of 588 white persons examined, 4·1 per cent., and of 1,938 natives examined, 5·7 per cent. had Vi serum agglutinins in a titre of 1 : 10 or higher. Three stool and urine cultures were made on all the white reactors without any success. A varying number of examinations were

made on forty of the native reactors, but typhoid bacilli were isolated from the urine of only two of them. Of 149 contacts of typhoid fever cases, 26 per cent. gave a positive Vi reaction, but in only 3 of them were typhoid bacilli demonstrated by culture—again in the urine.

On the whole, experience in Great Britain and the United States suggests that in the search for chronic carriers the Vi serum test is a most valuable preliminary measure. Very occasional carriers, infected perhaps with a strain of bacillus devoid of Vi antigen (see Rauss 1939), may have no Vi agglutinins; and some persons with a positive Vi serum test may prove persistently negative on culture. In such instances it is wise to examine the duodenal juice, since there is reason to believe that biliary carriers may excrete typhoid bacilli in the fæces intermittently, or in too small numbers to be detected by any of our present methods. Occasional Vi-positive carriers may be suffering from a bone lesion, and cannot be expected to show the presence of typhoid bacilli in the fæces or urine, though, of course, they may do so. Disappearance of Vi agglutinins from the blood of a carrier is suggestive of cure.

The technical demonstration of Vi agglutinins is most conveniently carried out by making use of a strain of typhoid bacillus that will respond only to Vi agglutinin in the serum (see Felix 1938*b*). Final serum dilutions should be put up at 1 : 5 onwards, the tubes should be incubated at 37° C., not at 50° C., for 2 hours, and the readings should be taken after a further 22 hours at room temperature. Living bacilli may be used, but the Oxford Standard suspension, made from killed organisms, is safer and more convenient; it does not usually prove satisfactory, however, for more than two months after its preparation.

In tracking down carriers of typhoid and of paratyphoid fever, whether transitory or permanent, and in establishing the relation of different cases to each other, the *bacteriophage method of typing* the bacilli is of very great service. As this subject has already been discussed in Chapter 30, we need do no more here than remind the reader that the susceptibility of a given strain to lysis by the bacteriophage is intimately associated with the Vi antigen, and that the different bacteriophage types are remarkably specific. For a description of the types of typhoid and paratyphoid bacilli prevalent in Great Britain and the United States, and of the value of the method in epidemiological investigations, the reader is referred to papers by Lazarus (1940), Felix (1943), Felix and Callow (1943), and Bradley (1943), and for a review of the carrier problem in general to the monograph by Browning and his colleagues (1933).

The Effect of General Sanitation on the Prevalence of Typhoid Fever.

Having discussed the part played by the chronic carrier of enteric infection it will be convenient to consider briefly the changes in the incidence of typhoid fever which have occurred during the last 50 years, and their relation to the improvements which have taken place, during the same period, in the sanitary organization of civilized communities.

One of the most striking phenomena in the public health annals of this period has been the steady decline of enteric fever as an important item in the mortality statistics. In 1870 there were 388 deaths from enteric fever per million living in England and Wales, and 233 deaths registered as "ill-defined." In 1880 the enteric deaths had fallen to 261 per million, and the "ill-defined" deaths to 58, the latter probably as the result of better diagnosis. In 1890 the enteric deaths had fallen to 179 per million, and the "ill-defined" to 13. In Table 136, for

which we are indebted to Miss Woods, are set out the death rates at ages from enteric fever in England and Wales, during the first 27 years of the present century. It will be noted that, for the first decade (1901–10), the death rate for males at the most susceptible age period (20–25) is no higher than for the population as a whole in 1890. Since 1910 the mortality from enteric fever has fallen rapidly; and in 1927 it had reached almost negligible proportions—less than 10 per million living.

TABLE 136

ENTERIC FEVER: ENGLAND AND WALES. DEATH RATES PER MILLION

	0–	5–	10–	15–	20–	25–	35–	45–	55–	65–	75 and up
Males:											
1901–10 .	32	52	71	137	179	178	142	104	80	40	17
1921–24 .	3	6	10	20	19	20	17	16	16	13	3
1925 .	6	5		16		14	10	9	11	9	4
1926 .	2	5		11		16	14	15	10	11	7
1927 .	2	8		12		11	12	14	12	5	—
Females:											
1901–10	30	59	81	100	99	97	86	67	50	29	10
1921–24	3	9	11	19	18	16	15	13	7	9	3
1925 .	1	8		12		12	13	11	12	7	7
1926 .	2	5		12		7	12	11	11	4	7
1927 .	4	3		11		9	10	14	12	6	4

This fall has not, however, been equally rapid in all Western countries. Gay (1918) points out that the mortalities in European cities during the decade 1901–10 varied from 17 per million in Stockholm to 337 per million in St. Petersburg. The average figure for the 33 largest European cities during this period was 65 per million, while the average figure for the 57 largest American cities during the last year of the decade was 195·9 per million.

These variations in place and in time are associated with characteristic differences in sanitary organization. As Gay points out, prior to 1880 or thereabouts, typhoid fever was a disease of cities, the incidence varying directly as the density of population, as exemplified by the figures for Munich and the surrounding district; these show the highest incidence in the city garrison, a somewhat lower incidence in the city as a whole, and a still lower incidence in the surrounding rural area. After this time, however, the sanitation of large cities rapidly and steadily improved, especially with regard to the provision of a pure water supply, and the efficient disposal of excreta and sewage. This improvement in sanitation has more than compensated for the density of population, and the result has been a shift in the incidence of enteric fever from the populations of large towns to rural communities, living under more primitive sanitary conditions.

Godfrey (1928) records some interesting figures with regard to the present incidence of enteric fever in New York City and State. In New York City itself the cases—not deaths—number about 23 per 100,000 living. In towns with populations of 10,000-20,000 the incidence is about 70 per 100,000, in towns with populations of 5,000-10,000 it is about 63, in towns under 2,000 about 48, and in very small towns and villages about 42. Thus, while enteric fever is less frequent in New York City than elsewhere in the State, it is at

the present time a disease of the small town, rather than a rural disease in the strict sense of that term. This is probably due to the fact that the small town has failed to keep its sanitary organization abreast of its growth; so that the water supply, sewage disposal, and milk control are inadequate to protect a community rapidly increasing in density of population.

Godfrey includes a valuable table showing the incidence of enteric fever at ages, and the percentage fatality in each age group. Information of this kind is not available for many communities, and we reproduce his figures in Table 137.

TABLE 137

INCIDENCE AND FATALITY OF ENTERIC FEVER IN DIFFERENT AGE GROUPS (after Godfrey).

Age Group.	Incidence (percentage of cases at all ages).	Case Fatality Percentage.
Under 5	4·5	8·4
5–9	13·8	4·4
10–14	14·3	6·4
15–19	14·2	10·2
20–24	12·0	12·5
25–29	9·7	13·5
30–34	7·7	16·4
35–39	6·3	15·2
40–44	5·4	15·2
45–49	3·9	19·9
50–54	3·3	22·9
55–59	2·0	22·8
60 and over	2·9	36·5

Enteric fever, at the present time, is thus a disease with its maximum incidence in adolescence; but the case fatality increases throughout life, save that children under 5 show a higher case mortality than children between 5 and 15.

In addition to the striking fall in the incidence of enteric fever as a whole, there was during the post-war years a change in the sex incidence, and in the prevalent bacteriological type of infection. Thus it was noted by Chauffard and his colleagues (1921), Weigmann (1926) and Knorr (1927) that after the middle period of the war the incidence of enteric fever was higher among women than among men, thus reversing the normal sex incidence, as universally experienced before the war. There can be little doubt that this shift was the result of the experience undergone by the male sex between 1914 and 1918. Thus, in an epidemic at Pforzheim in 1919 (Knorr 1927), the lower incidence among males than among females commenced at age 19 and ended at age 50, indicating very clearly the effect of military service. It seems probable that some part, at least, of the present advantage of the male sex was referable to the antityphoid vaccination of the troops during the war period; but there are facts which suggest that this is not the whole story. In Schleswig-Holstein, for instance, during the period 1914–24, the advantage of the males extended to enteric fever caused by *Salm. paratyphi B*, while the German troops, unlike the French and English, were immunized only against *Salm. typhi*. It is possible that this heightened average resistance of the male may have been due to mild or atypical infection while on war service, but the question must for the moment remain an open one.

Prophylactic Immunization against Enteric Fever.

Antityphoid vaccination in man was initiated by Wright in 1897 (see Wright and Semple 1897, Wright 1902, 1908)—though the observations of Pfeiffer and Kolle (1896) have been held by some to establish a claim to priority—and has since been tried under field conditions on the largest scale. The recorded results strongly

support the view that it significantly reduces the incidence of enteric infection in environments in which such infection is particularly likely to occur.

The most impressive figures are those collected by the Antityphoid Committee (Report 1913), referring to the incidence of typhoid fever among inoculated and uninoculated troops stationed abroad. These figures have been analysed in a valuable paper by Greenwood and Yule (1915), to which reference should be made by all those who are interested in the general question of the interpretation of data of this kind. Employing the method of the fourfold table, and including as inoculated all those who had received typhoid vaccine at the time of the last available return, the data are set out in Table 138.

TABLE 138

	Not Attacked.	Attacked.	Total.
Inoculated. . .	10,322	56	10,378
Not inoculated .	8,664	272	8,936
Total	18,986	328	19,314

$\chi^2 = 180{\cdot}38.$
P= less than 0·0001.

The value χ^2 is a measure, devised by Pearson, of the probability that the distribution actually observed might have arisen as the result of chance; as χ^2 increases, this probability decreases. The value P gives this probability in the usual numerical form (*i.e.*, in this particular case the odds are greater than 9,999 to 1—actually they are much greater —against the observed distribution having arisen as the result of chance).

War conditions provide an admirable breeding ground for typhoid and paratyphoid infections; and it might have been expected that 1914–18 would have yielded a final proof of the efficacy of antityphoid inoculation, and a close measure of its exact protective value. They did, in truth, greatly strengthen the presumptive evidence in its favour —we cannot reasonably suppose that the whole of the vast difference between the deadly rôle of typhoid in the Boer War (105 cases per year per 1,000 of strength) and its relative insignificance among the huge armies on the Western Front 15 years later (2·35 cases per year per 1,000 of strength—see Harvey 1929) was wholly due to better sanitary conditions. But the exigencies of war, the impossibility of getting complete figures for the inoculated and the uninoculated exposed to risk, and the fact that the proportion of inoculated men rose, in the British Army, to well over 90 per cent. by the end of 1915, rendered impossible the collection of mass figures of a kind suitable for detailed statistical analysis. The general trend of the evidence is not, however, in doubt. Thus (see Harvey 1929) the year 1915 provides figures for the percentage of inoculated men in the Army in France and the cases of typhoid in the inoculated and the uninoculated, from which the incidence in each group can be calculated. The incidence among the inoculated was 9·5 per 10,000, among the uninoculated 103·52 per 10,000. During this period the men were inoculated against typhoid only. The incidence rates for paratyphoid fever were, among the inoculated 21·53 per 10,000, among the uninoculated 39·84 per 10,000. This suggests some degree of group protection against paratyphoid infection—a result that would hardly have been expected on immunological grounds—but the wide difference in the incidence ratios (10·90 : 1 in favour of the inoculated in the case of typhoid and 1·85 : 1 in the case of paratyphoid) provides additional support for the view that the recorded figures do in fact represent a saving in morbidity due to the typhoid vaccine.

There is evidence, as might reasonably be expected, that the decrease in morbidity was associated with a decrease in case fatality, at least so far as typhoid fever was concerned. The case-fatality rate among 1,728 inoculated and infected men in the British armies in France was 4·57 per cent.; among 703 uninoculated men it was 18·35 per cent.

Curiously enough this finding was not paralleled in paratyphoid infection: among 1,357 infected men who had been inoculated with T.A.B. vaccine the case-fatality rate was 1·25 per cent.; among 2,694 men who had not been inoculated with T.A.B. vaccine it was 1·34 per cent. The difference in the case fatality of typhoid in inoculated and uninoculated men is borne out by the figures recorded by Pfeiffer (1922) for the German Army; 12–20 per cent. among the uninoculated, 2–3 per cent. among the inoculated.

Ancillary evidence, which may perhaps be adduced in support of the efficacy of anti-typhoid vaccination, is provided by the curious change in the sex incidence of enteric infection in the years following the war that has been referred to on p. 1550.

The results so far recorded were obtained with vaccines prepared from ordinary strains of typhoid and paratyphoid bacilli. The observations, however, of Felix and his colleagues on the Vi antigen of the typhoid bacillus (see p. 1525) pointed to the importance of selecting for vaccine preparation strains that were rich in this particular antigen, if the maximum protective effect was to be obtained. Perry, Findlay, and Bensted (1933, 1934*b*), who carried out experiments on mice, likewise reached the conclusion that only fully virulent smooth strains could be relied upon to induce a satisfactory degree of immunity. Immunization with an avirulent smooth strain was almost as ineffective as that with a frankly rough variant. They differed, however, in failing to observe the extreme degree of lability of the Vi antigen that had been described by Felix and Pitt (1934*a*). In fact, they found that, so long as the strain employed was of the fully virulent smooth type, an ordinary heat-killed vaccine preserved with 0·5 per cent. phenol proved fully effective. As a result of their work, the British Army vaccine has since been prepared from freshly selected smooth fully virulent strains of typhoid and paratyphoid bacilli, grown on agar for 48 hours at 37° C., washed off with saline, killed by heating for 1 hour at 53° C., and preserved with 0·5 per cent. phenol. It is standardized to contain 1,000 million *Salm. typhi*, and 750 million each of *Salm. paratyphi A* and *B* per ml. The usual dosage for an adult male consists of two inoculations subcutaneously of 0·5 and 1·0 ml. respectively at about 10 days' interval.

The value of T.A.B. vaccine prepared in this way received further confirmation during the second world war (Boyd 1943*a*).

Briefly, it was found that Italian prisoners of war taken in the first Libyan campaign, most of whom had been inoculated previously with an Italian T.A.B. vaccine, suffered from typhoid fever during the winter of 1940–41. All prisoners were therefore inoculated or re-inoculated with Italian T.A.B. vaccine. In spite of this, another wave of typhoid fever occurred in May to September, 1941, along with an outbreak of paratyphoid A fever during June to October. The British Army T.A.B. vaccine was therefore given in the usual two doses. The outbreak rapidly waned, and infection remained low all through the summer of 1942. With the influx in November and December, 1942, of fresh prisoners from the El Alamein battle, a fresh wave occurred. On the other hand, among the British prisoners of war in the Benghazi camp, though there were about 11,000–12,000 cases of dysentery in a total strength of 16,000–24,000, no cases of enteric fever were notified during the four months June to October, 1942. These two pieces of evidence: (1) the occurrence of endemic and epidemic enteric fever among the Axis forces, not controlled by the Italian T.A.B. vaccine, but apparently controlled and prevented by the British Army T.A.B. vaccine; and (2) the absence of enteric fever among British prisoners of war in spite of most unsanitary conditions and the presence of enteric fever in their captors—are difficult to explain except on the assumption that the British Army T.A.B. vaccine affords a high degree of protection against enteric fever.

This vaccine appears to be very effective in practice, but it does not as a rule provoke the appearance of circulating Vi antibody when given subcutaneously, though it is said to do so on intravenous inoculation (Climie 1942). Felix (1941), therefore, endeavoured to improve on it by preparing a vaccine in which the Vi antigen was subjected to less rigorous treatment than that which it receives in the heat-killed phenolized vaccine. This he succeeded in doing by killing the organisms with 75 per cent. alcohol and preserving them in 25 per cent. alcohol. Comparative trials of the two vaccines on human beings showed that, after administration of the alcoholized vaccine, Vi antibodies were demonstrable in a relatively high proportion of subjects, whereas after the ordinary vaccine they could seldom be found (Felix, Rainsford and Stokes 1941). The dosage recommended for the alcoholized vaccine is half that of the heat-killed vaccine, namely 0·25 and 0·5 ml. for an adult male, the interval between the two injections being preferably three weeks. The value of this vaccine has not yet been established in the field, but there is no reason to suspect that it will be less, and some reason to expect that it may be greater, than that of the heat-killed product. It has the advantage over this product of giving rise to rather less local, and considerably less constitutional reaction (Climie 1942, Report 1942*b*).

In South Africa an endotoxoid T.A.B. vaccine has been extensively used, prepared according to the method described by Grasset and Gory (1927*a*, *b*) and Grasset (1931, 1935). This consists essentially in washing off a 48-hour agar growth with distilled water, killing the organisms by heat at 58° C. for 30 minutes, breaking them up by four successive heatings and freezings, and finally rendering the endotoxin so obtained non-toxic by incubation for a month in the presence of 0·7 per cent. formalin. The endotoxoid can be given in larger doses than vaccines of whole bacilli. According to Grasset (1938*b*) the vaccine has been used for the inoculation of about 400,000 persons with more satisfactory results, judged by the diminution in the case incidence and case mortality rates, than those previously obtained from ordinary T.A.B. vaccine. Grasset's claims seem to be borne out by the experience of the South African troops in the North African campaign, which fared as well as those inoculated with the British Army vaccine (Boyd 1943*b*).

Kourilsky, Kourilsky and Boivin (1939) tried to make a vaccine from the pure glycolipoid somatic antigens of the typhoid bacillus, but were unable to reduce the toxicity of the preparation sufficiently. More recently, however, Morgan, Favorite, and Horneff (1943) report success in the preparation of a suitable purified antigen.

There is little exact knowledge on the *keeping quality* of ordinary heat-killed vaccine preserved with 0·5 per cent. phenol or tricresol, but so far as can be judged from their ability to stimulate the production of agglutinins in rabbits or to protect mice against infection with living organisms, vaccines stored at a temperature of under 10° C. seem to undergo little deterioration in potency over a period of three years (McCoy and Bengtson 1920, Perry, Findlay and Bensted 1934*a*, Mishulow, Mowry and Stocker 1937, Babudieri 1939).

Whatever vaccine is used it is desirable, if immunity is to be maintained at a high level, to re-inoculate subjects every 2 to 4 years. For this purpose one dose is sufficient. Siler and Dunham (1939) of the American Army recommend a single dose of 0·1 ml. (100 million organisms) of the phenolized vaccine injected intradermally; according to their findings, this provides an adequate stimulus, and gives rise to less local and constitutional reaction than the larger dose which is usually given subcutaneously. The alcoholized vaccine is not suitable for intradermal injection.

The *oral administration* of typhoid vaccine, which was suggested by the observations of Besredka (see Chapter 53), has been tried fairly extensively, but the results so far obtained have not been sufficiently encouraging to justify its substitution for the subcutaneous method (see de Mello *et al.* 1931, Grasset 1938*b*).

In the chapter on herd immunity we described experiments on mice; and arguing, with the necessary caveat, from mice to men, we drew the tentative conclusion that antityphoid inoculation, carried out by the best technique at present available, would form a poor substitute for the ordinary methods of sanitary control as applied to a population exposed to a severe and continuous risk of infection. There seems no reason to modify this view in the light of the recorded results on man. Inoculated men appear to do much better than inoculated mice, but they are never exposed to such severe and continuous risk. Even with their advantages in this respect there is no evidence that the inoculation of all those at risk will stamp out the disease, though it will greatly reduce both its incidence and its fatality. As a method of decreasing the chances of infection or death run by persons who are exposed to a serious but not overwhelming risk for a limited period of time, antityphoid inoculation must be regarded as a very valuable prophylactic measure. (For a review of immunization against typhoid fever, see Siler *et al.* 1941).

Prophylactic Vaccination during an Epidemic.

The beneficial effect on the morbidity rate of vaccinating contacts during the course of sporadic outbreaks of typhoid fever has been reported by Ramsey (1935). His observations have been quoted as a precedent for similar treatment of persons exposed to infection in water or milk-borne outbreaks of the disease. The two cases are not strictly parallel. There is reason to believe that vaccination during the incubation period of the disease may, instead of protecting, induce the opposite result, namely the so-called "provocation" typhoid. For this reason Topley (1938) has suggested that the use of passive immunization with antityphoid serum would be more appropriate for such persons. Possibly, combined active and passive immunization, such as has proved successful in the treatment of diphtheria epidemics (see p. 1401), might be even more satisfactory, but we must await the trial of this method before recommending it for general use.

The Treatment of Typhoid Fever with Antityphoid Sera.

There are various references in the literature to the treatment of typhoid fever by the injection of antisera prepared by immunizing horses with cultures or extracts of typhoid bacilli, but until recent years little success appears to have been achieved by this method. With the increase in knowledge, however, of the antigenic structure of the typhoid bacillus that resulted from the demonstration of the H, O and Vi antigens, and from the extraction of the O and Vi antigens in relatively pure form by chemical means, a way was opened up for the preparation of sera containing the desired antibodies and for checking the amount of individual antibodies in sera by suitable animal tests. Grasset (1930, 1931, 1938*a*), in South Africa, reported favourable results from the use of an antiserum prepared against the somatic antigen. In 1938 he stated that the serum had been tried on about 3,500 cases of typhoid fever, and had reduced the case mortality from 20 to 10 per cent. In cases in which it was administered during the first three days of the disease, the serum reduced the case mortality to 3 per cent. Felix (1935)

suggested the use of a serum containing Vi antibody in addition to the O antibody. Preliminary trials carried out in Palestine, in which a few control cases were treated with normal horse serum, yielded promising results. Favourable reports on the value of such a serum have since been published by Robertson and Yu (1936) at Shanghai, McSweeney (1937) at Dublin, Cookson and Facey (1937) and Hodgson (1944) in this country, Pijper and Crocker (1939) at Johannesburg, and Landor (1941) at Singapore. The serum should be given to adults in a dose of 33 ml. on two or three successive days. Intramuscular injection is the rule, but the intravenous route may be preferable for the first dose in severe cases. According to Felix (1935) the Vi antibody has an anti-infective action, suppressing multiplication of the typhoid bacilli; the O antibody, on the other hand, is chiefly responsible for neutralizing the endotoxin. This interpretation is not entirely borne out by the observations on mice of Grasset and Lewin (1937), Boivin and Mesrobeanu (1938*c*), and Longfellow and Luippold (1943), all of which suggest that the O antibody has some anti-infective power; but there is general agreement on the protective value of both antibodies. The mode of preparation of the serum has been described by Felix and Petrie (1938); and the method of titration and of assessment in terms of provisional units of Vi and O antibody has been laid down by Felix (1938*b*). The real therapeutic value of the serum will never be established until an adequate series of treated and control cases are observed together. Whether, in view of the favourable reports on the serum, it is too late to make such a trial, must remain open to question.

Chemotherapy in Enteric Fever.

Though Buttle and his colleagues (1937) found that the administration of sulphanilamide protected mice against multiple lethal doses of typhoid and paratyphoid bacilli, none of the sulphonamides has yet been shown in practice to have any beneficial action on patients suffering from enteric fever (Report 1943*b*). Numerous drugs have been tried for the treatment of chronic typhoid and paratyphoid carriers, but in spite of occasional favourable reports on the use of iodophthalein (Saphir, Baer and Plotke 1942) or of sulphaguanidine (Loewenthal and Corfield 1943), the medical treatment of these carriers has so far met with little success.

Enteric Infection in Animals.

It is impossible to consider in any detail the numerous varieties of enteric infection which are known to occur among the lower animals under natural conditions. References to the relation of the different species or types, within the typhoid-paratyphoid group, to various forms of animal infection will be found in Chapter 30. It may be noted that the bacterial species with which we have so far been concerned in this chapter are not natural pathogens of animals; while the species and types which produce enteric infection in swine, rats, mice, etc., usually give rise to acute gastro-enteritis when ingested by man (see Chapter 72).

The rôle played by *Salm. choleræ-suis* in hog cholera is discussed in Chapter 88.

Bacillary White Diarrhœa of Chicks.—References to several papers dealing with *Salm. pullorum*, the causative organism of this disease, and with the lesions which it produces in chicks and adult fowls, will be found in Chapter 30. A useful review of the literature is included in a paper by Rice (1926); and an account of the epidemiology of the disease, and of its control in flocks, is given by Dalling,

Mason and Gordon (1928). Apart from its great economic importance, the disease is of interest in that it affords an example of congenital infection *via* the egg, and an illustration of the application of bacteriological principles in the control of infective disease among live-stock.

The first cases of disease among an infected brood usually occur soon after hatching, and the outbreak often assumes epidemic proportions within a few days or weeks. Susceptibility to the natural spread of infection, in its severe form, would seem to be confined to the first few days after hatching. The incubation period is stated to vary from 4 to 10 days, though Rice records experiments in which it was as long as 28 days. Birds which survive the first few weeks seldom contract the disease in its acutely fatal form. Apart from the whitish diarrhœa, from which the disease derives its name, but which is by no means invariably present, the symptoms consist in drowsiness, thirst, and lack of appetite. The average duration of the disease is 2 or 3 days ; and the mortality is high—on the average about 70 per cent. At necropsy the lungs may be congested : the liver is enlarged, and may, according to some observers, show an unusual degree of mottling ; the intestine usually shows well-marked catarrhal enteritis.

Birds which survive the acute attack, and probably others which have never suffered from the typical disease, frequently develop a persistent ovarian infection ; and it is carrier birds of this type which perpetuate infection from one breeding season to another. A considerable proportion of the eggs laid by such birds are infected with *Salm. pullorum* ; Kaupp (quoted by Rice) records nearly 20 per cent. of infected yolks among 3,510 fresh eggs examined. Thus, in an infected flock, each year's hatchings produce a high proportion of infected chicks, and the cycle is repeated from year to year.

According to Severens, Roberts and Card (1944) genetic factors play an important part in determining susceptibility to the disease. During the first 5–10 days of life there is an increase in resistance to experimental infection, which is far greater in some chicks than in others. The increase is accompanied by a rise in the absolute and relative numbers of circulating lymphocytes. Evidence is brought to show that procedures such as splenectomy or irradiation with X-rays, which lower the lymphocyte count, lead also to a diminution in the resistance of the host.

The problem of control clearly resolves itself into the elimination of the carrier hens and this has been rendered possible by the application of the agglutination test. Blood is obtained from the wing vein, and the serum tested against suspensions of *Salm. pullorum.* This organism is non-flagellated, and contains only O antigens : the conditions must therefore be adjusted to reveal agglutination of this type. It is customary to use living suspensions, but it seems likely that alcoholized suspensions would prove as sensitive and could more readily be standardized. Incubation should be prolonged for 24 hours, though the results are usually readable after 4 hours at 50° C. The titre usually taken as diagnostic of infection is 1 : 40 to 1 : 50. Adopting this standard, it would appear that some 90 per cent. of carrier hens can be detected (Rice 1926). Such tests may be carried out at any time, and the detected carriers should be killed. The essential point, in preventing the perpetuation of infection from one season to another, is the elimination of all carriers among the birds preserved for breeding. The whole flock should be tested when the hatching season is over ; those which show agglutinins should be killed, and those which give negative reactions should be retested.

Only birds which have twice reacted negatively, and whose previous history gives no reason to suspect infection, should be retained for breeding in the following year.

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CHAPTER 70

BACILLARY DYSENTERY

Introductory

Together with plague, cholera, and influenza, dysentery has been one of the great scourges of the world. Though rapidly growing less common, it was at one time a fatal epidemic disease, dogging the footsteps of armies in the field, present in camp, gaol, and hospital, and decimating the white races in the tropics. "Wherever the general hygienic conditions are bad, wherever the soil is much fouled by excreta . . . , wherever many people are crowded together in one building or camp, where the food is coarse, monotonous or unsound, there, especially in tropical and sub-tropical climates," dysentery is liable to break out (Manson 1914).

Before the last quarter of the nineteenth century dysentery was regarded as a single, well-defined disease. In 1875, however, Loesch demonstrated the presence of parasitic amœbæ in the stools of dysenteric patients, and in 1883 Robert Koch observed these amœbæ in the intestinal wall of patients dying of dysentery in Egypt. The subsequent demonstration of amœbæ in liver abscesses of patients who were then suffering, or who had at some previous time suffered, from dysentery left little doubt that at any rate one of the causal agents of this disease was the *Entamœba histolytica*. In 1898–1901 Shiga in Japan, Flexner in the Philippines, and Kruse in Germany brought evidence to show that another type of the disease was due to a bacillus which was closely allied to *Salm. typhi*. Dysentery may also at times be caused by protozoa other than *Entamœba histolytica*, such as *Balantidium coli* and *Bilharzia mansoni* (see Manson-Bahr 1942).

In this chapter we shall deal exclusively with bacillary dysentery, referring our readers to textbooks on tropical medicine and protozoology for descriptions of the amœbic and balantidial types.

Historical.—Dysentery affords an illuminating example of a case in which the agglutination reaction was successfully employed to discover the causative agent of a disease.

Studying the epidemic of dysentery in Japan in 1896, Shiga (1898) isolated from the fæces and intestinal wall of dysenteric patients a Gram-negative, non-gelatin-liquefying coliform bacillus. This organism, which was cultivated from 34 patients, differed from the common *Bact. coli* in its failure to coagulate milk, and in certain other cultural properties. At the same time nine other organisms were cultivated. To ascertain which of them, if any, was causally related to the disease, Shiga tested the action of the patient's serum on each one of them. Only one of the types was agglutinated. This type was, moreover, never found in the fæces of patients suffering from other intestinal diseases such as typhoid or simple diarrhœa, nor was it agglutinated by the serum of such patients, or by the serum

of healthy persons. In the acute stage of dysentery it was present in almost pure culture in the blood-and-mucus stools; but during convalescence its isolation was difficult or impossible. This bacillus he called *Bact. dysenteriæ*. Though fatal to laboratory animals injected parenterally, it did not reproduce the characteristic symptoms of dysentery when given by the mouth.

In 1900 Flexner (1900*a*, *b*), then a member of the Commission sent out by the Johns Hopkins University to study the diseases prevailing in the Philippine Islands, reported the discovery of a bacillus closely resembling Shiga's.

A similar organism was discovered, also in 1900, by Strong and Musgrave working at Manila. In the same year Kruse (1900) in Germany described a bacillus that he had isolated from the fæces of 23 out of 24 patients suffering from dysentery.

There was at first considerable confusion with regard to the relation of these various strains to one another. This was finally elucidated by the investigations of Martini and Lentz (1902), who found that a serum prepared by the injection of a goat with Shiga's strain agglutinated Shiga, Kruse, and one of Flexner's strains (New Haven), but failed to react with Flexner's other strains (1 and Manila) or with Strong's bacillus. On the other hand, a serum prepared against Flexner I agglutinated Flexner I and Flexner Manila, but not Strong, Flexner New Haven, Shiga or Kruse. They likewise found that all of these bacilli were non-motile and non-flagellated. On a serological basis, therefore, they concluded that Shiga, Kruse, and Flexner New Haven were identical, and differed from Flexner I, Flexner Manila, and Strong; these two Flexner strains likewise differed from Strong's bacillus. This conclusion received striking confirmation from the further work of Lentz (1902); testing the sugar reactions of the dysentery bacilli, he was struck by the observation that Flexner I, Flexner Manila, and Strong all produced acid in mannitol, whereas Shiga, Kruse, and Flexner New Haven did not.

Lentz's work served to divide the dysentery bacilli into (1) the non-mannitol-fermenters—Shiga and Kruse; and (2) the mannitol-fermenters—Flexner I and Manila, and Strong. The former group was called the Shiga or the Shiga-Kruse group, the latter the Flexner group. (It is clear that Flexner isolated both the Shiga and Flexner types in the Philippines; but the one that bears his name is exclusively the mannitol-fermenting type.)

It will be noted that without the agglutination reaction it would have been extremely difficult to prove the ætiological rôle of the dysentery bacillus. This organism, unlike *Salm. typhi*, does not invade the blood stream, and cannot therefore be cultured from the blood. It does not reproduce the disease on injection into animals; and by cultural properties alone it is not easily distinguishable from closely allied organisms. The agglutination reaction, in conjunction with the invariable presence of this bacillus in the acute stages of epidemic dysentery, sufficed to differentiate it from other organisms and to indicate its pathogenicity to man.

Besides the Shiga-Kruse and the Flexner group, numerous other organisms have been found capable of giving rise to dysentery, such as *Sh. sonnei*, *Sh. schmitzi*, *Sh. newcastle*, *Sh. boyd*, and according to some workers members of the para-Shiga group (see pp. 1567–1569 and Chapter 29).

Epidemiology.

Bacillary dysentery is primarily an epidemic disease; but like most epidemic diseases it often occurs endemically. At present it is commoner in tropical than

in temperate climates, but this is largely due to lack of adequate sanitation and personal hygiene. In former times, when sanitary measures were not so well enforced as they are now, dysentery was widespread all over Europe; and in our own times it needed only a war with its accompanying indifferent sanitation to allow it to become rampant again. Wherever large numbers of men are in close contact for any length of time under poor hygienic conditions, dysentery may be expected. Thus it has always been, and to a certain extent still is, a disease of armies, gaols, hospitals, and asylums. Amœbic dysentery, on the other hand, occurs sporadically, and is mainly confined to tropical countries.

Though in the past recognition has been accorded almost solely to dysentery in its epidemic form, increasing attention has been paid of late years to the endemic disease. The careful study carried out by Bojlén (1934) in Denmark has shown that in that country the disease is widespread and of frequent occurrence. Similar conclusions have been reached in the United States as the result of surveys by McGinnes and his colleagues (1936) in Virginia, and Watt, Hardy and DeCapito (1942) in New Mexico, Georgia, and Puerto Rico. The real incidence of the disease is difficult to assess, since in most countries only the severe cases are notified. In Great Britain the reported cases rose from 683 with 155 deaths in 1928 to 4,167 with 139 deaths in 1937; but there is strong reason to believe that the actual number of cases was considerably in excess of these figures. As Bojlén (1934) in Denmark and Charles and Warren (1929) in this country have shown, the majority of the recognized cases occur in children. The sexes are attacked equally. The case mortality varies widely according to circumstances and particularly to age. It is especially high in mental hospitals, in times of famine, and in infections due to the Shiga bacillus. In the first three months of life it tends to be high, but it decreases gradually till about three years of age. In Uruguay, for example, Hormaeche (1940) recorded a case-fatality rate in infants under one year of age of 45 per cent., and Blacklock and Guthrie (1937) in Glasgow of 59 per cent. These figures relate to the severer cases that were admitted to hospital, and are not truly representative. For the population as a whole the case-fatality rate in frank cases of the disease in Denmark, Great Britain and the United States is probably about 2 per cent. (Bojlén 1934, Scott 1938, Weil 1943). In temperate zones the disease is commoner in the summer months, but the seasonal difference in incidence is relatively small. In sub-tropical and tropical countries, however, the effect of season is more striking. According to Rogers (1913), for example, bacillary dysentery in India is at a minimum in the cold months of January and February. With the onset of the hot weather in March there is a small rise, followed, however, by a fall during the very hot months of May and June. The maximum increase follows closely the beginning of the heavy monsoon rains in July, and the incidence reaches its highest point in August and September. With the cessation of the rains late in October and the fall of temperature, the incidence steadily declines.

It is of interest to note that the months when dysentery is most common are also the months when flies are most abundant. In this country Graham-Smith (1911–12) showed that non-lactose-fermenting Gram-negative bacilli could often be isolated from flies during the summer months when diarrhœa was prevalent, and that these insects could contaminate food over which they walked.

Working in Fiji, Manson-Bahr (see Bahr 1912) likewise obtained evidence that infection was partly carried by the house fly. The fly population was at its maximum when dysentery was most prevalent. Ample opportunity existed for con-

tamination of the food ; and *Sh. shigæ* was actually isolated from the lower intestinal tract of two flies caught on the bed of a patient suffering from acute dysentery. This suggestion was confirmed by the careful work of Taylor (1919) during the first world war. In the Salonika Expeditionary Force the maximum incidence of dysentery occurred when flies were most abundant. The conditions of temperature and humidity most favourable for fly breeding were present between the middle of April and the middle of June, and again between the middle of September and the end of October. Corresponding to this, the peak of the dysentery curve was reached at the end of May and at the end of October. During the hot dry weather of July, August, and early September, both the fly prevalence and the dysentery incidence curves fell practically to zero (Fig. 284). Experiments made

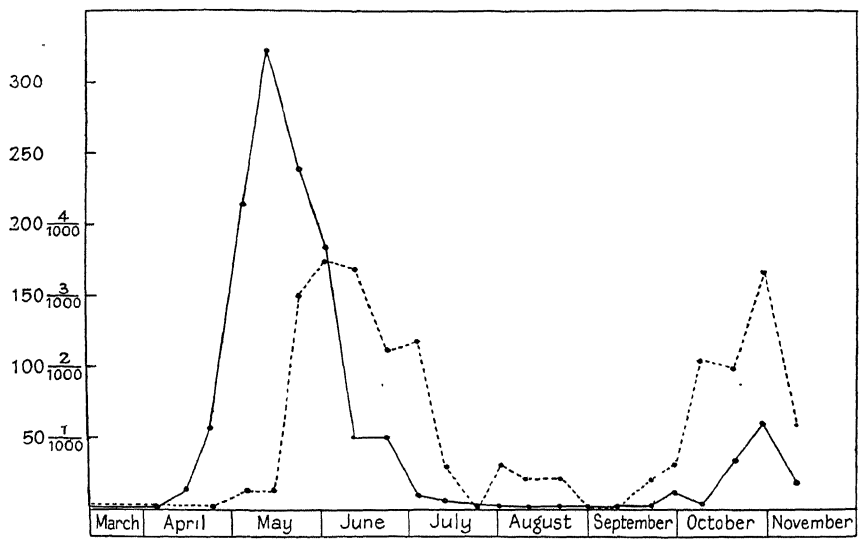


FIG. 284.

Showing the relationship between the prevalence of flies and the incidence of dysentery in the Salonika Expeditionary Force.

Continuous curve = Number of flies trapped *per diem*.
Interrupted curve = Incidence of dysentery cases per 1,000 *per diem*.
(After Taylor.)

to test the ability of flies to carry dysentery bacilli showed that these insects, after feeding on milk cultures or on dysenteric fæces, might remain infective for 24 hours. Of 1,670 flies caught under natural conditions, 1 from a hospital kitchen was carrying *Sh. shigæ*, and 8 were carrying Flexner's bacillus. Manson-Bahr (1919) likewise isolated *Sh. shigæ* from the lower intestine of flies in Palestine. It is doubtful whether under the modern sanitary conditions of Western civilization flies play more than a small part in the transmission of either the epidemic or the endemic disease. In the East and Middle East, however, as has been shown again in the experience of the second world war, fly-borne infection is of major importance (Fairley and Boyd 1943); and even in the United States it is not unknown under military conditions (Kuhns and Anderson 1944). Though dysentery bacilli may be found occasionally in the intestinal contents of these insects,

they do not appear to survive for more than a few days. The probability is that flies act mainly by carrying infective material on their feet rather than in their intestine.

Epidemic infection may result from the contamination of milk or of ice-cream; several well-authenticated outbreaks due to these vehicles have now been recorded in different countries. Under exceptional conditions the disease may be water-borne; but though one large outbreak in Japan due to an aberrant dysentery bacillus affecting 11,000 persons and causing 500 deaths (Report 1938), another large outbreak in Kansas due to *Sh. flexneri* affecting 3,000 persons (Kinnaman and Beelman 1944), and a smaller outbreak in this country affecting about 400 persons, in which *Sh. sonnei* was isolated from the water itself (Green and Macleod 1943), apparently arose in this way, the evidence does not suggest that epidemic disease is often determined by such means. In Java, however, where peculiar conditions prevail, bacillary dysentery is said to be predominantly a water-borne disease (Fairley and Boyd 1943). Food, especially of the ready cooked type, probably constitutes an important means of transmission.

Infection is derived as a rule from cases, particularly ambulant cases, of the disease, from healthy convalescents, from symptomless carriers—usually temporary—and probably far less often, except in mental institutions, from chronic carriers. The organisms, which are excreted in the fæces, may gain access to food through the imperfectly cleansed fingers of the patient or carrier; or they may pass from one person to another by contact with inanimate articles such as seats, door handles, and water-flushing devices in lavatories, bed-pans and washing bowls in hospital wards, pencils and books in schools, and crockery and glasses in restaurants. There are in fact so many ways in which infection may spread that, once it is introduced into nurseries or children's wards in hospitals, it is often extremely difficult to eradicate.

Bacillary dysentery varies greatly in its clinical manifestations and in its severity. In every outbreak there are a number of cases of simple diarrhœa, which are nevertheless caused by the dysentery bacillus. Entirely symptomless carriers are not uncommon, and in endemic areas may constitute a considerable proportion of those found to be infected (Watt and Hardy 1945). Even though the disease is often fatal in infants and very young children, it may be extremely mild and give rise to no more than a trivial disturbance of the bowel disappearing within 24 hours (Report 1943*a*). Occasionally food-borne outbreaks, particularly of the Sonne type, are characterized by acute gastro-enteritis rather than by true dysentery (Rohleder 1938, Report 1942*a*). A very small number of cases of dysentery that recover, about 2 to 3 per cent. according to Bojlén (1934), are said to suffer from chronic colitis. Among the few complications that may occur conjunctivitis is to be looked for about the tenth day of the disease and iritis during the second week; arthritis and neuritis are sometimes met with (Manson-Bahr 1942).

Asylum Dysentery.—Asylum dysentery is a particular form of the endemic disease occurring in a closed community. It is to Kruse (1901) that its recognition as an infectious disease was mainly due. In this country Mott (1901–02) made a careful study of the condition from its clinical and epidemiological aspects. More recently Bojlén (1934) carried out an extensive series of observations in Denmark. He found that, in contrast to endemic dysentery in the general population, asylum dysentery is characterized by a high case mortality, varying from about 10 to 20 per cent. Relapses, and probably re-infections, are very common,

and as many as 50 per cent. of the inmates may suffer from chronic dysentery. Persistent carriers are met with, and together with the numerous patients suffering or recovering from dysentery, they provide a continual source of infective material which is responsible for the further propagation of the disease. Spread of infection by carriers is of course rendered particularly easy owing to the low standard of personal hygiene among the insane.

Bacteriological Findings in Different Epidemics.—One of the striking features of the bacteriology of dysentery is that in any large epidemic it is almost invariable to find bacilli of more than one type. A pure *shigæ* or a pure *flexneri* epidemic on a large scale appears to be a rarity, though in small outbreaks a single type may alone be isolated (Kruse *et al.* 1907). Not only so, but in any large outbreak, besides the well-defined Shiga and Flexner bacilli, it is usual to isolate a number of other organisms. In the past these have been referred to as para-Shiga, in-agglutinable Flexner, or more generally para-dysentery bacilli. Our increasing knowledge has served to classify many of these organisms into Schmitz, Sonne, Boyd and Newcastle types. There remain, however, a number of strains whose exact classification is at present doubtful, and whose rôle in the causation of dysentery still awaits elucidation. Where the disease is endemic, only one or two types may be concerned, and in institutions only a single type may be found.

In different epidemics, in different countries, and at different times, the proportion of cases due to different bacillary types varies considerably; and even in the same epidemic the results of different observers may show a marked dissimilarity in this respect. In the British Salonika Force, Dudgeon (1919) and his co-workers obtained the following distribution of organisms in bacteriologically positive cases of dysentery (Table 139):

TABLE 139

	No. of Cases.	Percentage.
Sh. shigæ	335	38·3
Sh. flexneri Y	503	57·6
Other Types	36	4·1

That is to say, Flexner's bacillus was half as common again as *Sh. shigæ*. But Graham (1918) and his co-workers, who investigated 2,500 cases of dysentery and diarrhœa in the British Salonika Force at much the same time—June, 1916, to the end of 1917—found that of the severe and moderately severe bacillary dysenteries 75 per cent. were due to *Sh. shigæ* and only 25 per cent. to *Sh. flexneri Y*, whereas of the mild bacillary dysenteries 75 per cent. were due to *Sh. flexneri Y* and only 25 per cent. to *Sh. shigæ*.

The figures given by Boyd (1936) in India are more complete (Table 140). They show that just over half the strains isolated were of the classical Flexner type, and that about 13 per cent. belonged to newly recognized Flexner or Boyd types; Shiga's bacillus constituted about 15 per cent., Schmitz's bacillus 5 per cent. and Sonne's bacillus 9 per cent. of the total strains studied.

In the United States Shiga's bacillus seems to be uncommon. Hardy, Watt, Kolodny and DeCapito (1940), who isolated 2081 strains of dysentery bacilli from cases of diarrhœa and dysentery in New Mexico during the years 1936, 1937, and 1938, did not meet with it once. They identified 65·0 per cent. of their strains

as *Sh. flexneri*, 19·1 per cent. as *Sh. sonnei*, and 15·9 per cent. as *Sh. newcastle*. Shiga's bacillus is, in fact, seldom met with except in tropical countries and East Asia (Weil 1943).

TABLE 140

TYPES OF DYSENTERY BACILLI ISOLATED IN INDIA 1932 TO 1934 (MODIFIED FROM BOYD 1936)

	No. of Strains.	Per cent.
Sh. shigæ	820	14·94
Sh. schmitzi	296	5·39
Sh. newcastle (Type 88)	240	4·37
Sh. flexneri Types V, W, X, Y, Z	2,888	52·61
„ „ Type 103	135	2·46
„ „ Type P119	100	1·82
Sh. boyd Type 170	134	2·44
„ „ Type P288	26	0·47
„ „ Type P274	47	0·86
„ „ Type D1	41	0·75
„ „ Type D19	5	0·09
Inagglutinable mannitol fermenters	57	1·04
Mannitol fermenters not fully investigated	95	1·73
Sh. sonnei	508	9·25
Inagglutinable lactose and sucrose fermenters	97	1·77
	5,489	99·99

Clinically the disease due to Shiga's bacillus is much more severe than that due to Flexner's; as well as the abdominal pain, diarrhœa and tenesmus, there is often a profound toxæmia ending in collapse. Complications such as arthritis (Graham 1919, Report 1919) and iridocyclitis, when they do occur, are almost invariably associated with the Shiga type of infection. Moreover, recovery from dysentery due to *Sh. shigæ* is a slow process; frequently the patients remain debilitated for months, and on attempting to resume work are liable to relapse (Fletcher and Mackinnon 1919). This corresponds with the result of animal experiments, which show that Shiga's bacillus is much the more toxic organism of the two.

Besides the historically well-known Shiga and Flexner types, a number of other types have been recognized of late years. Some of these play a very important part in the causation of dysentery, particularly of the endemic and institutional variety, and require attention.

Schmitz's Bacillus.—This was described by Schmitz (1917), and called *B. ambiguus* by Andrewes (1918). It was first encountered (with the possible exception of an American outbreak, see Park and Carey 1903) as the cause of an epidemic of dysentery in a Roumanian Prisoners of War Camp. The morbidity rate was high —10 per cent.—but the deaths were few. The bacillus, which, it will be remembered, differs from *Sh. shigæ* in producing indole, was isolated from a number of the patients. It was agglutinated by their serum to a titre of 1/500 to 1/1,000; Shiga's bacillus was likewise agglutinated to much the same titre, but Flexner's bacillus only to between 1/200 and 1/500. Apparently the bacillus was able to give rise to the production of group agglutinins, which were especially active towards *Sh. shigæ*. Incidentally it is interesting to remark that the epidemic occurred in the depth of the very severe winter of 1916–17, and indeed reached its

maximum in January of the latter year. There is little doubt from Schmitz's account that the insanitary conditions prevailing in the camp were sufficient to explain the spread of the disease. This bacillus has now been found in other countries (see Mandry 1935, Boyd 1935, 1936). Of late years it has been reported mainly from institutions, especially for mental patients. Schleifstein and Coleman (1937), for example, recorded an outbreak affecting 200 persons in a New York State institution; and in this country reports of cases in mental institutions have come from Evans (1938) in Wales, from Knox in Leicester (Report 1942c), and from Shera (1943) in Eastbourne. It is rarely responsible for more than a minority of cases in any given area.

Para-Shiga Bacilli.—Attention was drawn by Dudgeon and Urquhart (1919) to the occurrence among dysentery cases in Macedonia of non-mannitol-fermenting bacilli that failed to agglutinate with a Shiga antiserum. Since then, numerous authors have made similar observations, such as Large and Sankaran (1934) and Sachs (1943) in India, Ali (1938) in Egypt, and Hazen (1938) in the United States. Some of these so-called para-Shiga bacilli produce indole, others do not. They differ also in arabinose fermentation and in certain other ways. Sachs (1943) recognized eight different types, all antigenically distinct. At Quetta, where he looked for them carefully, they constituted 7 per cent. of the dysentery strains isolated. They were found mainly in the first three days of the disease, and were absent from control patients. As a rule agglutinins appeared in the serum of the patient, reaching a titre of 1/250 or 1/500; no agglutinins were found in a series of 200 Wassermann sera examined. Of the para-Shiga strains isolated, 93 per cent. came from cases of acute dysentery, in which no other type of dysentery bacillus could be demonstrated. In view of these observations, and of others so far unpublished, there is little doubt that para-Shiga bacilli may give rise to clinical dysentery.

Sonne's Bacillus.—This is a more important organism. Though it had undoubtedly been previously observed in the United States and Germany, it was Sonne's description in 1915 that drew serious attention to it. From a number of sporadic cases of dysentery in Denmark Sonne cultivated a bacillus differing from *Sh. flexneri* in its late fermentation of lactose, in its marked production of acid in litmus milk during the second week of incubation, and in its different serological reactions. In an investigation of dysentery in Norway, Thjøtta (1919) found that 25 out of 65 strains isolated conformed to the Sonne type. More recent work in Denmark (Bojlén 1934) has shown that *Sh. sonnei* is as common as *Sh. flexneri* in the causation of endemic and institutional dysentery. In this country the organism has been often encountered (see Channon 1926, Kerrin and Cruickshank 1926, Fyfe 1927, Hay 1930, Cann and de Navasquez 1931, MacGill and Downie 1932, Pickles 1932, Nabarro and Signy 1932, Laws 1936, Carter 1937, Thornton and Darmady 1938). It seems to be very common in the United States and Canada (see Johnston and Brown 1930, Nelson 1930, Leahy 1931, Felsen and Osofsky 1934, Weil 1943), and has been observed in several other parts of the world. It is most frequently met with in endemic dysentery, particularly in asylums, children's hospitals, and nurses' homes. In infants it may give rise to an acute toxic form of dysentery, which in Japan is known as *Ekiri*. The disease may, however, occur in epidemic form, and several milk-borne outbreaks have been described (see Sylvest 1933, Bojlén 1934, Felsen and Osofsky 1934, Bowes 1938, Faulds 1942, 1943). Leuchs and Heim (1930) report an outbreak involving 51

persons due to infected cheese. Clinically, Sonne dysentery is said by some observers to be less severe than Flexner dysentery, but Bojlén (1934) disputes this. Mild and atypical cases of the disease are common in any reasonably large outbreak. As has already been mentioned, Sonne's bacillus often gives rise to acute gastro-enteritis rather than to dysentery proper. Rohleder (1938), who analysed 118 cases in which this bacillus was demonstrated, found that 72 of them had run a clinical course indistinguishable from that of a simple gastro-enteritis. Bacteriologically, the organisms are found in the fæces, and occasionally in the urine (Rohleder 1938); agglutinins are often demonstrable in the blood serum of patients suffering or convalescent from the acute disease. The closely related organism, *Sh. dispar*, has so far not been definitely incriminated as a cause of dysentery, but further observations are required (see Forsyth 1933). The fact that some indole-positive strains were isolated by Felsen and Osofsky (1934) and Beck and Buckle (1939) from hospital outbreaks of dysentery suggests that this organism cannot be dismissed as a possible cause of the disease.

Newcastle Bacillus.—This organism was first recognized as a cause of dysentery by Clayton and Warren (1929*a*, *b*) in Newcastle. Downie, Wade and Young (1933) in Manchester described a similar organism, differing in the fermentation of mannitol; and Boyd (1936) in India described under the term Type 88 another organism that fermented mannitol but produced no trace of gas in glucose. Whitehead and Scott (1934) pointed out the relationship between these three organisms. The Newcastle bacillus, in one or other of its variant forms, has now been isolated from many parts of the world. In the series of 2,081 strains of dysentery bacilli studied by Hardy, Watts, Kolodny and DeCapito (1940) in New Mexico, it constituted no less than 15·9 per cent. of the total.

Shigella flexneri Types 103 and P119.—Type 103 formed 4·1 per cent. and P119 2·7 per cent. of 4,856 strains of mannitol-fermenting dysentery bacilli studied by Boyd (1938) in India during the years 1932 to 1934. From the evidence presented by Boyd (1931, 1936) there seems little doubt that both these organisms, which are antigenically related to *Sh. flexneri*, are capable of giving rise to dysentery.

Shigella boyd Types 170, P274, P288, D1.—Types 170, P288, and D1 are believed by Boyd (1939–40) to be responsible for some cases of dysentery. In India they comprised 3·9, 1·3, and 1·4 per cent. respectively of the series of 4,856 strains alluded to above. P274, whose pathogenicity seems to have been established by Rothstadt, Fenner and Baker (1942), comprised 1·5 per cent.

Shigella alkalescens.—That this organism is occasionally pathogenic to man there seems little doubt. It was isolated from the blood stream of a woman suffering from puerperal fever (Smith and Fraser 1928), and was encountered in an institutional outbreak of food poisoning (Welch and Mickle 1934). It has been cultured from the fæces of sporadic cases of dysentery, chronic enteritis, and diarrhœa (see Brown and Anderson 1936); but it seems to be found most frequently in infections of the urinary tract (Snyder and Hanner 1937, Neter and Heide 1940). Like many other non-lactose fermenters it is commoner in the inflamed than in the normal intestine, and its mere presence cannot therefore be regarded as establishing its pathogenicity.

The rôle of Morgan's No. 1 bacillus—*Proteus morgani*—is likewise doubtful. Douglas and Colebrook (1917) found it in 29 per cent. of patients who had contracted

dysentery in Gallipoli, while Candler and Dean (1911) isolated it from 5 out of 16 patients suffering from asylum dysentery. It seems to be an organism that is capable of multiplying in the intestine under suitable conditions, but it can hardly be ranked along with the Shiga, Flexner, and Sonne types as a primary cause of dysentery.

Dysentery Carriers.—Patients recovering from an acute attack of dysentery may continue to excrete the bacilli in their stools. Generally this excretion lasts for only a week or so, but a small proportion become persistent carriers.

Fletcher and Mackinnon (1919), who during the war of 1914–18 had unrivalled opportunities for studying the carrier condition in a depot for dysentery convalescents, found that of 935 patients 71 or 7·59 per cent. were carriers, and that 26 or 2·78 per cent. were persistent carriers. (They defined " persistent carrier " as one who carried the bacilli for more than 3 months after the beginning of the disease.) Of the 71 carriers 58 were carriers of Flexner's and 13 of Shiga's bacillus. Between these two classes there were striking differences. All the *shigæ* carriers belonged to the persistent group, whereas only 13 of the *flexneri* carriers were persistent excreters. The *shigæ* carriers excreted the bacilli with greater constancy than did the *flexneri* carriers; thus out of 469 samples of fæces from *shigæ* carriers the bacillus was isolated 207 times, or once in every 2·26 specimens; whereas of 1,803 samples from *flexneri* carriers the bacillus was found only 330 times, or once in every 5·46 specimens. The *flexneri* carriers had a tendency to be intermittent; the bacillus was excreted for one or two days in succession, and then not again for a month or more; it was then excreted once more, only to disappear again after a few days. Even more striking was the clinical difference between the two series. The *shigæ* carriers looked ill and suffered from chronic dysentery and extreme mental depression; the *flexneri* carriers, on the other hand, were in moderately good health and were comparatively cheerful. This is to be attributed in part at least to the frequent relapses to which the *shigæ* carriers were subject; no sooner had their diarrhœa ceased and their general condition improved, than the slightest dietetic indiscretion or resumption of exercise or work brought on again an acute attack of the disease.

The conclusions to be drawn from these observations are that dysentery due to *Sh. shigæ* is altogether a more serious condition than that due to *Sh. flexneri*, and that its liability to become chronic renders it a cause of prolonged invalidism. Both classes of carriers are, however, a danger to the community, and every practicable step should be taken to avoid their spreading the disease.

Studying institutional dysentery in Denmark due to *Sh. flexneri* and *Sh. sonnei*, Bojlén (1934) was struck by the frequency of persistent carriers. Some patients, whose stools were systematically examined, were found to excrete the bacilli for years. The experience of most workers, however, shows that chronic carriers—that is, those who excrete the organism for a year or more—are infrequent, and therefore play but a small part in the spread of the disease, except perhaps in mental institutions.

It is sometimes stated that there are no healthy carriers of dysentery bacilli, and that all those who excrete the organism are suffering from chronic dysenteric lesions (see Scott 1938). The evidence in favour of this view is far from convincing. Several workers have demonstrated the presence of *Sh. flexneri*, *Sh. sonnei*, *Sh. schmitzi*, and even *Sh. shigæ* in completely symptomless carriers (see Report 1942*b*, *d*, Weil 1943). It is possible that before the introduction of desoxycholate citrate medium, cultures were successful only from heavily infected persons; but now that even small numbers of dysentery bacilli may be demonstrated, the existence of perfectly healthy carriers is no longer in doubt. Hardy, Watt and

DeCapito (1942), for example, found that in an endemic area as many as 3·8 per cent. of persons who gave no history of illness during the preceding year were "passive carriers" of the dysentery bacillus. Doubtless they had been in contact with other carriers or cases, and had become infected without developing the disease.

The introduction of S.S. and desoxycholate media has also led us to modify somewhat our ideas on intermittency of excretion. Even so, it is not uncommon to observe intermittency in convalescents and carriers, though less often than formerly (see Cruickshank and Swyer 1940, Report 1942*e*). The apparent duration of excretion has been extended by the use of these media. Most workers would have been prepared to accept Fletcher and McKinnon's (1919) findings that not more than 2 to 3 per cent. of convalescents carried the bacillus for over three months. Watt, Hardy and DeCapito (1942), however, working with S.S. and with desoxycholate citrate media, found that 11 per cent. of Flexner, 10 per cent. of Newcastle, and 7 per cent. of Sonne carriers excreted the bacillus for over ten weeks.

In conclusion it is worth while comparing dysentery with other enteric infections, notably typhoid. Dysentery has a short incubation period, about 48 hours as a rule. It is essentially a local disease ; the bacilli remain confined to the intestine and to the mesenteric glands ; they do not invade the blood stream, nor are they, except occasionally, found in the spleen or liver. In this respect dysentery—of the *shigæ* type—has been compared to diphtheria ; the bacilli produce a necrosis of the intestinal mucosa, often with pseudo-membrane formation, and their toxic products are absorbed, giving rise to profound systemic disturbance. The disease may also run a chronic course. Typhoid fever, on the other hand, has a long incubation period—up to 21 days ; it is primarily a septicæmic disease with secondary localization in the intestine ; the bacilli do not produce a soluble exotoxin ; and the disease has little tendency to become chronic.

Laboratory Infection in Man.—It has been pointed out in Chapter 29 that, with the possible exception of monkeys, dysentery cannot be reproduced in laboratory animals. There are, however, a few definite cases recorded in the literature of experimental infection in human beings. Kruse (1901) mentions the case of Dr. Stöcker, an assistant in his laboratory at Bonn, who apparently infected himself while working with cultures of Shiga's bacillus. He suffered from a mild attack of the disease ; the bacilli were isolated from his stools ; and a typical rise and subsequent fall were observed in his blood agglutinins. For several years previously there had been no dysentery in the town. Flexner (1900*b*) likewise records the case of a laboratory assistant at Baltimore, who aspirated a fluid culture of the Flexner Manila strain into his mouth. He spat it out and rinsed his mouth with weak phenol, but within 48 hours he had severe diarrhœa, pain, and tenesmus, with blood and mucus in his stools. Unfortunately no cultures were made.

Diagnosis.

Blood and urine cultures are almost invariably sterile. In the bacteriological diagnosis of dysentery most stress should be laid on the isolation of the specific organism from the fæces, less on the presence of agglutinins in the patient's serum. The fæces should be examined as early as possible in the disease. The highest percentage of positive results is obtained from blood-and-mucus stools (Table 141).

TABLE 141

SHOWING PROPORTION OF ISOLATIONS OF DYSENTERY BACILLI FROM DIFFERENT TYPES OF FÆCES (Report 1919).

Total number of cases examined = 3,125.

	Blood and Mucus.	Diarrhœal Stools plus Mucus.	Diarrhœal Stools.
Total Number Bacteriologically examined.	1,103	741	1,281
Positive	664 or 60·2%	160 or 21·6%	50 or 3·9%
Negative	439 or 39·8%	581 or 78·4%	1,231 or 96·1%

The best results are obtained when the blood and mucus are examined within an hour of being passed, before other organisms have had time to proliferate. If this is impossible, the mucus or fæces should be collected into a buffered 30 per cent. glycerol saline solution, such as that described by Sachs (1939), which prevents the dysentery bacilli being destroyed by the acid produced during the growth of other organisms. Rectal swabs are often of value in acute cases, or when special care has to be exercised to avoid substitution, or, in hospitals, in order to avoid contamination of the sample through imperfectly sterilized bed-pans.

Microscopical examination of the mucus in the early stage of acute bacillary dysentery shows an extreme degree of cellularity, 90 per cent. or more of the cells, apart from red blood corpuscles, being polymorphonuclears. In the later stages the number of cells decreases, the pus cells become degenerate and proportionately fewer, the red blood corpuscles are scanty or absent, mononuclear cells increase, and the exudate, in coming to resemble that found in amœbic dysentery, loses its diagnostic significance.

Cultures should be made, preferably from washed flakes of mucus, on Leifson's (1935) desoxycholate citrate agar or on the so-called S.S. medium, both of which are very much superior, especially in the examination of convalescents and contacts, to MacConkey's or Endo's medium (see Hardy *et al.* 1939, 1942, Hynes 1942, Pot 1942, Report 1942*f*). The brilliant green method, which is of such value in the isolation of *Salmonella*, is of no help in the cultivation of dysentery bacilli, since these organisms are as susceptible to the dye as *Bact. coli* (Krumwiede and Pratt 1914, Myers and Koser 1932). Colonies should be picked off into lactose broth, put through the sugars, and tested by the tube or the slide method against specific agglutinating sera of the types most likely to be met with in the area concerned. Slide agglutination must be used with care, particularly for colonies off selective media, since non-specific results are common. Shiga's bacillus on first isolation may be poorly agglutinable; heating for 1 hour at 60° C. is said to improve it (Schütze 1944). It should also be remembered that Flexner's bacillus may contain somatic antigens of the *Salmonella* group. A motility test, preferably at 22° C., should never be omitted (Braun and Weil 1928).

Agglutinins appear in the patient's serum about the 6th to the 12th day, but sometimes not till later, or not at all. The serum should be tested against standard suspensions of the common types of dysentery bacilli. The tubes are best incubated in a water-bath at 50° C. for 4 to 6 hours, left overnight at room temperature, and read finally the next morning.

Much less attention can be paid to the presence of serum agglutinins in the diagnosis of dysentery than in that of enteric fever. This is partly because normal

human serum may contain agglutinins for dysentery bacilli, and partly because the group agglutinins may be so prominent as to render the diagnosis of the type of bacillus almost impossible. The value of the test depends to a considerable extent on the frequency distribution of normal agglutinins in the population. In endemic areas and in mental institutions in which dysentery is common, interpretation of low serum agglutinin titres is very difficult and often impossible (see Dudgeon 1919). On the other hand, in areas in which dysentery is uncommon, and especially in children, whose serum is usually devoid of normal agglutinins, the test may be of value. Under these conditions a titre of 1/25 or over to *Sh. shigæ*, *Sh. schmitzi*, or *Sh. sonnei*, and a titre of 1/100 or over to *Sh. flexneri* is highly suggestive of active infection. Except to *Sh. flexneri*, the agglutinin titre seldom rises high, and often declines in early convalescence. In Sonne infections only about 60 per cent., as a rule, of patients develop serum agglutinins (Smith and Fraser 1930, Johnston and Brown 1930, Nabarro and Signy 1932, Laws 1936, Cruickshank and Swyer 1940), so that, while a positive result may be diagnostic, a negative result does not exclude the presence of active disease. In chronic cases agglutinins may persist for a long time.

Diagnosis at autopsy should be made by taking cultures from the mucosa and submucosa of the affected part of the intestine, and from the corresponding mesenteric glands. (For a fuller description of the diagnosis of bacillary dysentery see Report 1920.)

Prophylaxis, Vaccination and Serum Treatment.

Prophylaxis.—The general prophylaxis of bacillary dysentery is so similar to that of typhoid fever that there is no need to consider it in detail. Particular attention should be paid to the extermination of flies, and to the protection of food from both flies and dust. Persons who have had dysentery should not be permitted to act as cooks, or to have anything to do with the preparation of food.

If an outbreak of dysentery occurs in an institution, attention should be concentrated on the mode of preparation and distribution of food and on the prevention of direct and indirect cross-infection. The kitchen staff and all those in any way concerned in the handling of food should be carefully questioned for a history of current or recent intestinal disturbance, and should be instructed to report directly to an officer who is solely responsible for the control of the outbreak. Intensive examination of the food personnel for the presence of carriers, some of whom may be entirely symptomless, is to be recommended provided adequate laboratory facilities are available. Repeated samples of stools are usually required in order to detect fresh cases of infection and intermittent excreters. Bacteriological examination may be extended to the whole institution, but as a rule it is sufficient to restrict this to the food personnel and to segregate all other persons, whether staff or patients, who have a history of diarrhœa, dysentery, or chronic colitis (see Bojlén 1934). In hospital wards, however, bacteriological control is often necessary if infection is to be eradicated in the shortest possible time. Particular attention should be paid to the disinfection of lavatory seats, door handles, and water-flushing devices. Bed-pans are a fertile source of cross-infection in wards, and should be sterilized by boiling, steaming, or immersion in disinfectant after the contents have been evacuated. Under field conditions the fæces themselves must be burnt or disinfected (see Fairley and Boyd 1943), but in institutions with a satisfactory conservancy system this is seldom required. Though it

often presents practical difficulties, the rule should be enforced that no nurse handling bed-pans should have anything to do with the preparation or distribution of food. Each patient should have his own crockery and feeding utensils, which should be disinfected after use. All persons in the institution must be instructed to wash their hands thoroughly in a disinfectant solution, such as one containing 0·1–0·2 per cent. of active chlorine, after defæcation. In mental hospitals convalescent and chronic carriers probably play a large part in perpetuation of the infection, and must be sought for and suitably controlled.

Vaccination.—The value of vaccination in the prophylaxis of dysentery is still undecided. There are two main difficulties. Firstly, there are so many different types of dysentery bacilli that any vaccine to be effective would have to be polyvalent. Secondly, owing to the great toxicity of Shiga's bacillus, the preparation of a suitable vaccine has so far proved impracticable.

Numerous attempts have been made to overcome this latter difficulty by modifying the toxicity through the addition of tricresol (Gay 1902), eusol (Dean and Adamson 1916), hydrogen peroxide (Dean and Adamson 1916), formalin (Dumas, Ramon and Bilal 1926), or soft X-rays (Moore and Kersten 1936). Shiga (1903) advocated the use of a sero-vaccine, the first dose of which was followed in 4 days by an inoculation of bacilli alone. Antibodies were demonstrable in the blood of inoculated persons 3 to 4 weeks later. This method was used on about 10,000 persons in Japan during the years 1898–1900, and is said to have had little effect on the morbidity, but to have decreased the mortality considerably. Unfortunately Shiga gave no exact figures of the results. Olitsky (1918) has suggested the use of a lipo-vaccine consisting of heat-killed bacilli suspended in neutral almond oil, on the principle that, owing to slower absorption of the toxin, the patient's reaction should be correspondingly less. Iguchi, Ohstubo and Eguchi (1933) have tried oral vaccination with apparently satisfactory results; these, however, still await confirmation. More recently Boivin and Mesrobeanu (1938) have recommended the use of a glycolipoid antigen extracted from Shiga's bacillus, and Morgan and Schütze (1943) a phenol extract of the somatic antigen. No satisfactory or convincing evidence has yet been brought to show that in practice any of these vaccines is able to contribute significantly to the prophylaxis of Shiga dysentery. Formol toxoid is said by O'Brien (1939–40) to be a good antigen for horses, but it seems doubtful from the work of Steabben (1943) whether it could be relied on to protect against infection with living bacilli.

The difficulty of preparing a polyvalent vaccine against 6 or 12 types of dysentery bacilli and ensuring a satisfactory antigenic contribution from each of them is obviously considerable. Scott (1938) suggests for institutional use in this country a vaccine containing 150 million per ml. of each of the five common serological types of Flexner bacilli together with the same number of Newcastle and Sonne bacilli. He advises a dosage of 0·5 to 1 ml. according to age, sex, and physical strength, the vaccine being given on admission and repeated annually. Though private reports are often favourable, it must be remembered that rigidly controlled observations on alternate vaccinated and control patients are seldom made; and until more information is available it is probably wise to suspend judgment on the prophylactic value of dysentery vaccines.

Serotherapy.—Todd (1904) working in London, and Rosenthal (1903, 1904) working in Moscow, showed independently that the soluble toxin contained in cultures of *Sh. shigæ* was able to give rise on injection into animals to a true neutralizing antitoxin. Todd found that the serum had definite protective properties; if it was injected intravenously into a rabbit, it protected the animal

against a lethal dose of toxin or of dead bacilli, given simultaneously or half an hour later. A horse serum that he prepared contained sufficient antitoxin per ml. to neutralize 20,000 lethal doses of toxin for a rabbit. The union of toxin and antitoxin appears to take place in constant proportion (Pfeiffer and Ungermann 1909, Kolle *et al.* 1924).

Antiserum is prepared by the intravenous injection of horses, first with heat-killed and later with living bacilli (Flexner and Amoss 1915). More recently dysentery anatoxin has been used for the production of serum (Dumas *et al.* 1926).

The titration of the serum is best carried out on the mouse, using intravenous inoculation (Kolle *et al.* 1924, Sudmersen *et al.* 1924). O'Brien and Runge (1925) suggested as a provisional standard that the unit of antitoxin should be that amount of serum which would neutralize the test dose—0·2 mgm.—of a standard toxin; the test dose was approximately 10 lethal doses for the mouse. An improved method was described by Blake and Okell (1929), which according to Trevan (1929) leads to an increased accuracy in titration. Zozaya (1932) has used the polysaccharide precipitation test for the antibacterial standardization of serum, but Kurauchi and Nagata (1935) have reported unfavourably on this method.

Under the Therapeutic Substances Act one unit of Shiga antiserum is contained in 1/200 ml. of a 1 per cent. solution of a standard dry (Copenhagen) serum.

In practice the results of serum treatment are not very convincing. Shiga (1901), in Japan, tested the therapeutic effect of an antidysentery serum, the method of preparation of which is not recorded; from his report we have constructed the following table (Table 142).

TABLE 142

SHOWING MORTALITY OF PATIENTS IN HOSPITAL TREATED WITH MEDICINE ALONE OR WITH SERUM.

Year.	Hospital.	Mode of Treatment.	No. Treated.	Died.	Mortality.
					Per cent.
1895–99 . . .	Municipal	Medicine	2,565	929	36·2
1899	,,	Serum	88	11	12·5
1897	Infectious	Medicine	34	8	23·5
1898–1900 . .	,,	Serum	210	20	9·5

Unfortunately the figures of the serum-treated cases are few; moreover the comparison, so far as individual hospitals are concerned, has been made between the mortality of cases treated with serum in one year and that of cases treated without serum in previous years. Shiga concludes, however, that serum treatment reduced the mortality to about one-third of its former value. (For records of results obtained by other authors previous to 1907 see Deane 1907.)

During the first world war Graham (1918) treated a number of cases occurring in the British Salonika Force with a serum prepared against both Shiga and Flexner strains, and recorded favourable results in the relief of abdominal pain and tenesmus. During the second world war Fairley and Boyd (1943) had the opportunity of trying the effect of highly potent protein-digested anti-shigæ serum containing 20,000 I.U. per ml. Its use was restricted to proved Shiga cases. It was given intravenously in a dose of 5 to 10 ml., and repeated daily when thought advisable.

No serum reactions were observed in a series of over thirty cases. Though clinical improvement sometimes followed, it was often only transient. The authors formed the opinion that the serum was of temporary benefit by neutralizing toxin, but failed to modify the course of the disease on account of its lack of antibacterial activity. This view is largely borne out by the animal experiments of Felsen and Osofsky (1938), O'Brien (1939–40), and Steabben (1943). Fairley and Boyd would restrict the use of Shiga antiserum to fulminating or severely toxic cases immediately on admission to hospital, and to cases in which chemotherapeutic treatment has failed. The dosage should be not less than 200,000 I.U. intravenously, repeated in 12 hours if necessary.

On the whole, therefore, we may conclude that treatment with antitoxic serum to the Shiga bacillus is of limited and transient value. Its usefulness in practice is further curtailed by the inability to decide by clinical inspection alone whether a given patient is infected with the Shiga bacillus, or with some other type of dysentery bacillus. No evidence is available to show that a serum prepared against either the Flexner or the Sonne bacillus is of any therapeutic value (O'Brien 1939–40).

Treatment with *bacteriophage* has been tried. Some workers, such as Compton (1942), are convinced of its efficacy, but the findings are not sufficiently conclusive to demand detailed attention (see Riding 1930, Weil 1943, Fairley and Boyd 1943, Boyd and Portnoy 1944).

Chemotherapy.—Treatment of dysentery cases with drugs of the sulphonamide group is now being extensively used, and, though caution is necessary in interpreting the results (see Boland 1943), there is a general consensus of opinion that it constitutes a valuable form of therapy. There is still no unanimity on the best form of drug to employ. Sulphapyridine has been favourably reported on by Paulley (1942) and Swyer (1943), but most workers prefer sulphaguanidine on account of its lower toxicity and its lesser tendency to give rise to renal deposits. Fairley and Boyd (1943) recommend for acute cases an initial dose of 0·1 gm. per kilo of sulphaguanidine, a maintenance dose of 0·05 gm. per kilo 4-hourly so long as the number of stools exceeds five a day, and a further maintenance dose of 0·05 gm. per kilo. every 8 hours until the stools have remained normal for two days. The duration of treatment should not exceed 14 days. In many cases a lower dosage might well suffice. More recent work has pointed to the superiority of succinyl sulphathiazole (sulphasuxidine) and of sulphadiazine. The dosage for an adult of succinyl sulphathiazole need not exceed 10 gm. daily, divided into five doses (see Smyth *et al.* 1943, Report 1943*b*, Roberts and Daniels 1943). Sulphadiazine is recommended by Hardy, Burns and DeCapito (1943) for acute cases and succinyl sulphathiazole for convalescents and latent carriers. In very severe cases due to the Shiga bacillus it is well to combine chemotherapy with serum treatment. On the whole the available evidence seems to show that treatment with adequate doses of sulphaguanidine, sulphadiazine, or succinyl sulphathiazole cuts short the acute symptoms of the disease and lessens the chance of the patient's becoming a convalescent or chronic carrier. Once the carrier state is established, the value of sulphonamide treatment appears to be less. It is, however, always worth trying, and is often successful in Flexner and Shiga infections (Rantz and Kirby 1942, de Candole 1943); Sonne carriers appear to be more refractory, though favourable results have been reported with succinyl sulphathiazole (Brewer 1944).

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CHAPTER 71

INFECTIVE ENTERITIS OF INFANCY

THE title of this chapter suggests that there is an infective and a non-infective enteritis of infancy, and that the two diseases can be distinguished. The first suggestion is doubtless correct; but though some cases are certainly infective and others are almost certainly not, there is a substantial modicum of cases between these two extremes that cannot be assigned with any assurance to one or other group. Our knowledge of the causative factors underlying much of the enteritis of infancy is very incomplete; and all we can do in this chapter is to discuss certain types of enteritis which, either on bacteriological or on epidemiological grounds, appear to be infective. Since no satisfactory classification can yet be made on an ætiological basis we shall, for convenience of description, divide the cases in a provisional and arbitrary manner into the following groups, realizing that, in opposition to all the canons of classification, the groups themselves are not mutually exclusive.

1. Neonatal diarrhœa.
2. Primary infective enteritis due to known pathogenic organisms.
3. Primary infective enteritis due to, or associated with, organisms of doubtful pathogenicity—summer diarrhœa.
4. Enteritis following the consumption of food containing toxic substances of bacterial origin.
5. Infective enteritis of protozoal origin.
6. Infective enteritis, secondary to or associated with parenteral infection.

Epidemiology.—Though each of the six groups just mentioned has its own peculiar epidemiology, it will be well to give a picture of the disease as a whole. No satisfactory data exist on the incidence of infantile enteritis, either of the infective or of the non-infective type. It is probable, however, that the mortality figures, on which we shall have to rely, comprise mainly the infective cases. In considering the disease we shall not restrict ourselves entirely to infants under one year of age, but shall at times extend our survey to include children up to five years of age.

It will be seen from Table 143 that the death rate from diarrhœal diseases in infants fell considerably during the first 30 years of the present century, so that in the 1931–35 quinquennium it was only about 15 per cent. of what it was at the close of the last century. Since 1930, however, the rate has remained more or less stable.

Table 144 shows that the great majority of fatal cases occur during the first year of life, and that about 95 per cent. of deaths under five years of age occur

TABLE 143

AVERAGE ANNUAL DEATH RATE FROM DIARRHŒAL DISEASES IN INFANTS UNDER 1 YEAR OF AGE PER 1,000 LIVE BIRTHS. ENGLAND AND WALES.

Years.	Average. Annual Death Rate.	Years.	Average. Annual Death Rate.
1896–1900 . . .	31	1921–25	8
1901–05. . . .	23	1926–30	6
1906–10. . . .	18	1931–35	5
1911–15. . . .	19	1936	5
1916–20. . . .	9	1937	5

during the first two years. The footnote shows that the death rate is higher in males than in females.

Table 145 records the deaths from diarrhœa and enteritis according to the month of the year. Though deaths at all ages are included, it will be realized

TABLE 144

DEATHS AT AGES FROM DIARRHŒA AND ENTERITIS. ENGLAND AND WALES.

Year.	Age in Years.						
	0–1.	1–2.	2–3.	3–4.	4–5.	0–5.	All Ages.
1911	31,899	6,901	1,097	380	185	40,462	46,212
1916	2,391	702	144	48	21	3,306	14,041
1921	11,705	1,924	320	154	102	14,205	17,086
1926	5,394	969	201	129	77	6,770	8,415
1931	3,284	447	99	55	48	3,933	5,222
1936	3,220	260	74	37	25	3,616	4,990
1937	3,086	296	61	34	29	3,506	4,925

NOTE.—During these years 55·8 per cent. of the deaths under 5 years of age were in males.

from the previous table that the majority of them refer to deaths during the first two years of life. It is evident that in the hot year of 1921 there was a striking seasonal incidence, the deaths during the months of August, September and October exceeding those during the other nine months of the year. Though detailed figures are not provided in the Registrar-General's report, it is known

TABLE 145

DEATHS FROM DIARRHŒA AND ENTERITIS ACCORDING TO MONTH OF THE YEAR. (ENGLAND AND WALES. ALL AGES)

Year.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1921	942	775	793	614	600	581	1,591	3,880	3,066	2,306	1,162	763
1926	585	457	436	473	461	442	569	896	1,546	1,309	655	579
1931	538	377	472	399	407	380	390	403	433	461	476	476
1936	398	396	463	382	407	339	397	400	481	522	401	393
1937	355	328	386	351	427	373	408	433	470	459	441	490

that in the hot year of 1911, when there was a very high diarrhœal mortality, the majority of the deaths likewise occurred during the same period. Since 1921, in spite of seven hot years, the gross excess of deaths during the late summer and early autumn has not been witnessed again, and there is now little evidence of seasonal variation in the death rate (see Graham-Smith 1939).

Most authors stress the protective influence of breast-feeding. Niven (1904), for example, in Manchester, found that of 88 infants under one year of age suffering from summer diarrhœa, all but four were artificially fed. Dudfield (1912) in Paddington estimated that in 1911 the attack rate of breast-fed infants was 28·3 per cent., and of those artificially fed 58·6 per cent.; in the former group not a single infant died. Peters (1910) brought evidence to show that infants in the first year were more susceptible when artificially fed than those in the second year; we may conclude, therefore, that breast-feeding is responsible for delaying the onset of the disease. Further evidence of the protective effect of breast-feeding was contributed by Grulee, Sanford and Schwartz (1935) in the United States, who analysed the morbidity and mortality statistics relating to 20,000 infants, and found that the incidence of gastro-intestinal disorders was considerably higher in the artificially than in the breast-fed group. Several other authors could be quoted to the same effect. Without wishing in any way to belittle the importance of breast-feeding, we may point out that it is hardly fair to form an estimate of the superiority of breast over artificial feeding by merely comparing the incidence or mortality of diarrhœa in the two groups of children. As Dr. A. Bradford Hill pointed out to us some years ago, there is a fallacy in assuming that the two groups are strictly comparable. The mere fact that an infant is artificially fed often means that it was not thriving at the breast. The artificially fed group therefore must contain a proportion of infants suffering from disturbed digestion of one sort or another, and it is in consequence not surprising that these should be more prone to develop enteritis than infants who are responding perfectly well to breast-feeding. Other factors, too, such as social status and environment, must tend to upset the comparison in favour of the breast-fed group. If alternate children under exactly similar conditions could be reared on the breast and on an artificial diet, it is probable that the mortality from enteritis would be lower in the breast-fed group, but the difference between the two groups would almost certainly be considerably less than that indicated by most of the figures quoted. Breast-feeding is beneficial, partly because human milk is on the whole more easily digested than cows' milk; and partly because breast milk is not subject to contamination from fingers, flies and other sources to which liquid cows' milk is often exposed, and to which the devastating outbreaks of summer diarrhœa that occurred in the early years of the present century can be largely ascribed.

Social and environmental factors play a part in the epidemiology of infantile enteritis. Broadly speaking, the lower the social class, the greater the degree of overcrowding in the homes, and the dirtier the domestic conditions, the commoner is the disease. Poorly nourished babies are more likely to be affected than those which are well nourished. The disease is particularly prevalent in hospitals and institutions, where it constitutes a serious hazard to life. Dobbs (1941), for example, goes so far as to say that gastro-enteritis is the greatest menace to infants suffering from pyloric stenosis admitted to English hospitals. Premature infants nursed in hospitals, particularly if they are not breast-fed, are likewise peculiarly subject to the disease.

Neonatal Diarrhœa

This disease, which is sometimes referred to as epidemic diarrhœa of the newborn, has attracted attention only during recent years. Rice, Best, Frant and Abramson in 1937 were able to collect reports on eight outbreaks, but since then numerous others have been described in the United States and Great Britain. Our information, however, about the disease is still fragmentary, and its ætiology remains obscure. Almost all the reported outbreaks have occurred in maternity homes; but it is quite possible that sporadic cases occur which are not recognized as being of the same nature as the epidemic disease. Infants are said to be attacked chiefly in the first fortnight after birth, but again too much weight should not be laid on this statement, because this happens to be the usual length of stay in a maternity home. Infants developing the disease after they have left the home will often be overlooked. Ormiston (1941), describing one outbreak, mentions that when, for various causes, infants were kept in hospital for longer than the usual 10- to 14-day period, 46 per cent. were older than a fortnight when first affected. On the whole, however, the evidence does suggest that the disease is restricted to infants under one month of age, and that the commonest age at the time of onset is 5 to 8 days. There is no apparent difference in sex incidence; and though infants of the poorer classes are said to be more susceptible than those of the wealthier classes, social distinctions do not appear to be of great importance (Frant and Abramson 1938). Reports on the relative incidence of the disease in breast-fed and artificially fed infants are conflicting (Rice *et al.* 1937, Best 1938, Baker 1939, Forbes and Olsen 1939, Cron *et al.* 1940, Ormiston 1941). Many authors have observed no difference between the two groups; but this may perhaps be partly explained by the common custom in maternity hospitals of giving glucose water or some similar drink to all babies, thus affording a possible means of spreading infection. It is difficult to estimate the true morbidity of this disease.

Rice, Best, Frant and Abramson (1937) say that among 3,672 babies born alive in eleven lying-in institutions in New York City between July, 1934, and December, 1936, 505 are known to have contracted neonatal diarrhœa—a morbidity rate of 14 per cent. Among these the case-mortality rate was 46 per cent. Greenberg and Wronker (1938), referring to a New York hospital, mention a morbidity rate of 40 per cent. and a case-mortality rate of 29 per cent. In Great Britain Ormiston (1941) described outbreaks in three geographically separated institutions, and observed among 996 exposed infants a morbidity rate of 14 per cent. and a case-mortality rate of 29 per cent. In Sakula's (1943) series of 25 cases, no fewer than 20 deaths occurred.

Most authors agree on the high case-mortality rate, which may reach even 80 per cent., but the high morbidity rates just quoted must be understood to refer only to institutions in which the disease occurs. So far as can be ascertained from reported figures, the disease is unknown in the majority of maternity hospitals, so that its total incidence is probably low. There is little to suggest any particular seasonal prevalence; outbreaks occur in the winter as well as the summer. The incubation period may be as short as 2 days or as long as 20 days; the commonest period seems to be 2 to 6 days. In any given nursery there may be two or three sporadic cases, followed by a rapid succession of cases, giving to the outbreak an almost explosive suddenness; or there may be small groups of cases separated by intervals of a week or more.

This is not the place to describe the symptomatology of the disease. Briefly, however, the chief manifestations are a sudden or insidious onset, vomiting, slight

fever, diarrhœa, rapid dehydration and loss of weight, often followed by death in 1 to 7 days. Blood, mucus or pus in the stools are uncommon. At autopsy, the findings vary greatly. Often there is little to be seen besides congestion of the intestinal mucosa and slight swelling of the mesenteric lymph nodes. Sometimes there are hæmorrhagic pneumonia and acute hæmorrhage splenitis (Colvin and Emory 1941); and sometimes cerebral œdema and petechial hæmorrhages in the brain and viscera (Lyon and Folsom 1941, Bloch 1941). Acute enteritis may be found (Cron, Shutter and Lahmann 1940), but usually this is conspicuous by its absence.

Bacteriologically, with the exception of a single outbreak described by McKinlay (1937) in which a salmonella—later identified by Schiff and Strauss (1939) as *Salm. wichita*—was isolated from the stools, the findings of different workers have been unilluminating. The possible causation of the disease is discussed by Crowley, Downie, Fulton and Wilson (1941), who are unable to reach any definite conclusions. Many workers favour a virus infection, supporting this by the apparent relationship of some outbreaks in infants to the occurrence of epidemic influenza in the general population; others regard as a more probable cause some normal inhabitant of the intestine which is virulent for infants but not for older children. The general picture of the disease suggests an acute toxæmia, but what the nature or origin of the toxin is remains obscure. Light and Hodes (1943) claim to have demonstrated the presence of a filtrable agent in the stools, capable of giving rise to acute enteritis in calves; but their work awaits confirmation.

Spread of the disease is difficult to control by ordinary measures. Usually it is best to close the maternity home to further admissions, remove infected infants to suitable isolation quarters, discharge apparently healthy infants, and then disinfect the whole premises as thoroughly as possible.

Primary Infective Diarrhœa due to known Pathogenic Organisms

Observations, particularly during recent years, have rendered it evident that some cases of enteritis during infancy and early childhood are due to infection with members of the *Shigella* or *Salmonella* group. How frequent these are, it is impossible to say. In the past many cases have probably not been recognized, owing to imperfect bacteriological technique; but now that increasing attention is being drawn to these organisms, and highly selective media are available for their cultivation, they should be diagnosed more frequently.

Hormaeche (1943) who, with his colleagues, conducted a very careful investigation into the enteritis of infancy in Uruguay, found that of 668 cases of enteritis, 260 (38·9 per cent.) were due to *Shigella* and 152 (22·8 per cent.) to *Salmonella*; in the remaining 256 cases (38·3 per cent.) no pathogenic organisms could be demonstrated. The incidence of dysentery and salmonella infections is probably much lower in this country than in Uruguay; but that it is not negligible is shown by the fact that Blacklock and Guthrie (1937), working at the Royal Hospital for Sick Children, Glasgow, investigated during the years 1931–36 no fewer than 215 cases of dysentery in children up to 12 years of age, and Guthrie and Montgomery (1939), at Glasgow, investigated, apparently during the years 1937 and 1938, 28 cases of *Salmonella* infection in infants under two years of age. In Syria, Moore and Dennis (1940) studied 543 attacks of enteritis occurring in 490 infants and children, and found that 65 were due to dysentery bacilli, 11 to proved *Salmonella* strains, and 51 to strains that gave the correct sugar reactions of the *Salmonella* group but were not serologically identified.

Both dysentery and salmonella infections are said to be commoner in children than in infants. Whether this is so or not is doubtful. What does seem to be clear is that the case mortality is much higher during the first two years of life than later (Table 146).

TABLE 146

CASE MORTALITY OF ENTERITIS ACCORDING TO CAUSE AND AGE.
(From Hormaeche, 1940)

Age in Years.	Shigella Infections.		Salmonella Infections.		Cause Unknown.	
	Cases.	C.M. %.	Cases.	C.M. %.	Cases.	C.M. %.
0–1	51	45·1	39	30·7	135	28·9
1–2	41	31·7	20	15·0	39	23·1
2–3	18	5·6	11	0·0	9	33·3
3–12	16	0·0	10	0·0	6	0·0

C.M. = Case Mortality.

Pathologically, the disease tends to be more of an ileo-colitis than a gastro-enteritis. This is manifested clinically by the frequent occurrence of dysenteriform symptoms, and by the presence of blood, mucus and pus in the stools. Less often, the upper part of the intestine is attacked; the diarrhœa may then be of the frequent watery type, leading to rapid dehydration—the so-called cholera nostras or cholera infantum. Except in rare instances dysentery bacilli remain confined to the gut, but salmonella bacilli often invade the blood stream, giving rise to septicæmia or meningitis (Hormæche, Peluffo and Aleppo 1940). The commonest types of dysentery bacilli are *Sh. flexneri, Sh. sonnei* and *Sh. newcastle.* Of the *Salmonella* group, numerous members have been encountered, notably the *typhi-murium, newport, enteritidis, dublin* and *derby* types.

Bacteriological diagnosis is made in the usual way. The organisms should be cultivated from the fæces, using desoxycholate citrate agar for the dysentery group, and this medium, together with preliminary enrichment in brilliant green tetrathionate or selenite broth, for the *Salmonella* group. Sometimes *Salmonella* may be isolated from the throat, the middle ear, the cerebrospinal fluid or the blood. Serum agglutinins appear in a considerable proportion of the cases, particularly in older children, and are best demonstrated 2 to 3 weeks after the beginning of the disease (Hormaeche, Surraco and Aleppo 1939, Hormaeche, Peluffo and Aleppo 1940).

Primary Infective Enteritis due to, or associated with, Organisms of Doubtful Pathogenicity

This group contains the great mass of cases of unknown ætiology. In the last century and the early years of this century it was particularly prevalent during the late summer and early autumn months—the so-called summer diarrhœa of infants—and was responsible in hot years for a large number of deaths. Now, the summer excess is no longer seen, but the cases that occur throughout the year remain equally unexplained and puzzling.

Summer Diarrhœa.—Briefly, this disease used to be common both in Europe and in America. In hot summers it attained epidemic proportions. It was most

frequent in the second year of life, but the case mortality was highest 3 to 6 months after birth, when it sometimes reached a figure of 30–40 per cent. Breast-fed infants were comparatively exempt. The children of the poorer classes, living in hot, dirty, overcrowded tenements, suffered most severely. Its infectious nature was suggested by the occurrence of multiple cases in the same household (Niven 1904, Peters 1910, Sandilands 1910, Hutt 1914).

Ballard (1889) drew attention to the importance of temperature in the genesis of the disease. In his own words: "The summer rise of diarrhœal mortality does not commence until the mean temperature recorded by the 4-foot earth thermometer has attained somewhere about 56° F., no matter what may have been the temperature previously attained by the atmosphere or recorded by the 1-foot earth thermometer." He pointed out, too, that the maximal diarrhœal mortality of the year was usually observed in the week in which the 4-foot earth thermometer recorded its maximum, and that the decline of the diarrhœal mortality coincided with that of the 4-foot earth temperature, which lagged well behind the fall in atmospheric temperature. Influenced by Pettenkofer's doctrine, Ballard believed that the rise in earth temperature favoured the multiplication of some micro-organism, which infected the food and thus gained access to the alimentary canal. Later workers regarded the undoubted effect of temperature in a different light. Niven (1910), for example, working at Manchester, found a close correspondence between the prevalence of flies captured week by week and the incidence and mortality of diarrhœa. Hamer (1908) in London and Hope (1920) in Liverpool confirmed Niven's observations, but pointed out that the two curves were not always parallel (see Fig. 285). In 1916, 1917 and 1918 the two curves agreed closely; but in 1919, though flies were as prevalent as in the three previous years, the curve of diarrhœal mortality was very low.

Besides favouring flies and the consequent infection of food, a high temperature may cause a disturbance of the normal secretory mechanism of the infant's intestine. Arnold (1928), for example, brings evidence suggesting that it diminishes the amount of acid secreted by the stomach, and thus interferes with the gastro-duodenal auto-disinfecting mechanism, which is responsible for keeping the upper part of the intestine relatively free from bacteria. In addition, a high temperature promotes the multiplication of bacteria in milk and similar foods. This factor will clearly be operative to a greater extent in poor-class houses, ill provided with the means of keeping foodstuffs clean and cool, than in the houses of the well-to-do.

The bacteriological findings in summer diarrhœa have been most diverse. They were reviewed in some detail in the 2nd edition of this book (pp. 1250–53). Suffice it to say here that members of the dysentery group, *Proteus morgani*, *Proteus vulgaris*, *Ps. pyocyanea*, *Cl. welchii*, and certain other organisms were all incriminated by workers in different countries; but that no one single pathogenic organism was demonstrated by everybody. The only common finding was that non-lactose-fermenting organisms were greatly increased in the fæces; what the majority of these organisms really were remains in doubt. The bacteriological investigation of summer diarrhœa was carried out at a time when our technical methods were far less developed than they are now, and when several members of the *Shigella* and *Salmonella* groups were unknown. It is fairly clear that no one organism could have been responsible for all cases. We are therefore driven to the conclusion that summer diarrhœa, though appearing epidemiologically to be a well-defined disease, was in fact associated with a multiplicity of bacterial agents.

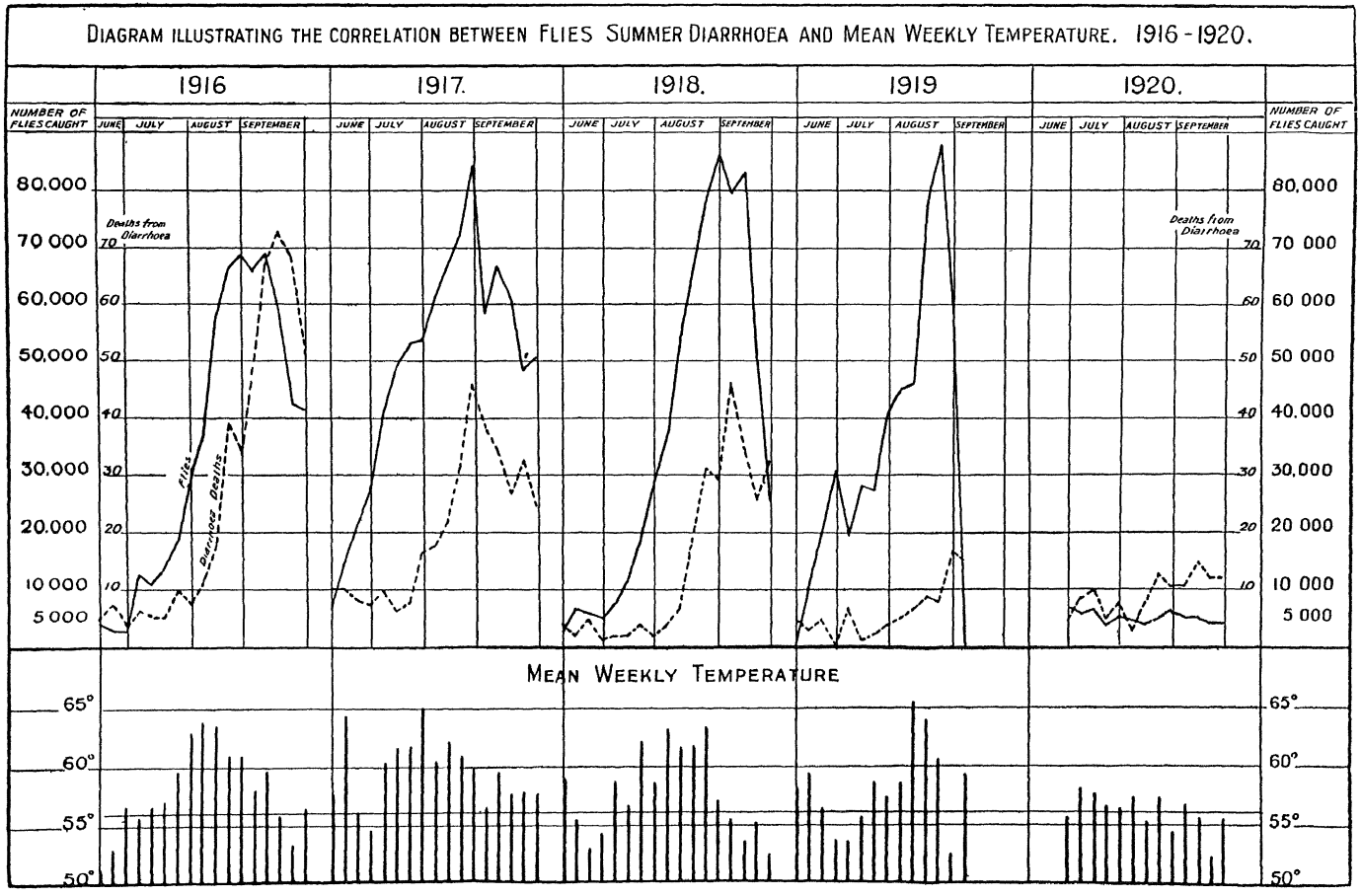


FIG. 285.—AFTER HOPE.

On the whole it seems probable that the disease was a bacterial infection, the prevalence of which was determined by a number of factors, varying to some extent independently, but all conditioned directly or indirectly by the prevailing temperature.

During the past 20 or 30 years, summer diarrhœa has practically disappeared from this country (see Table 145). The better disposal of garbage, the diminution of dust, the replacement of horse by motor traffic, the decrease in the number of flies, the improvement in cleanliness of the milk supply, and the greatly increased use of pasteurized, boiled and dried milk in the feeding of infants and children, have probably all aided in lessening the chances of bacterial contamination of food with pathogenic bacteria.

Enteritis following the Consumption of Food containing Toxic Substances of Bacterial Origin

Little is known of this group. It is included in Lyon's (1940) classification of the diarrhœal diseases of children; but owing to the peculiar difficulty of demonstrating bacterial toxins concerned in food poisoning, other than that formed by *Cl. botulinum*, progress in its study has necessarily been slow. Some strains of staphylococci are known to give rise under favourable conditions to an enterotoxin capable of causing acute gastro-enteritis in adults. The toxin may be formed in milk, and it is therefore almost certain that infants and young children fed on raw cows' milk must be affected from time to time. Whether other common organisms proliferating to a prodigious degree in milk can render the milk toxic to infants, either by virtue of their own body substance or by breaking down some substrate in the milk, is still doubtful. Many years ago Park and Holt (1903) brought evidence to show that milk containing large numbers of coliform bacilli, streptococci and micrococci, i.e. bacteriologically dirty milk, was able to give rise to gastro-intestinal disturbance in infants, but the exact mechanism by which this occurs has so far baffled inquiry (see Chapter 93).

Infective Enteritis of Protozoal Origin

There is considerable reason for believing that the flagellate, *Giardia lamblia*, may give rise to enteritis in infants and young children. This organism hooks itself on to the cells of the duodenal and upper ileal mucosa and, if present in sufficiently large numbers, seems to give rise to inflammation of the gut and possibly to interference with fat absorption (Véghelyi 1938, 1940). Moore and Dennis (1940) in Syria, whose observations have already been referred to, found it in 84 out of 543 attacks of enteritis. In infants, when blood was present in the diarrhœal stools, it was found in 22 per cent. of cases examined, as against only 10 per cent. in all cases of infantile diarrhœa. The difficulty in determining its pathogenicity lies in the circumstance that it is often found in the complete absence of symptoms, and that its increased frequency in the stools in the presence of diarrhœa may be explained by the more rapid passage of the organisms through the gut in an unaltered condition. The clinical evidence, however, in favour of its pathogenicity is fairly strong, and the disappearance of symptoms after treatment with mepacrine lends added weight to this view (see Ormiston, Taylor and Wilson 1942). The type of enteritis to which it appears to give rise may be acute, and be characterized by vomiting and diarrhœa, sometimes with mucus and traces of blood in the stools; but more often it is mild and chronic and is

characterized by 3 or 4 copious, loose, evil-smelling motions a day. The disease is seldom fatal.

Mepacrine has the same specific action on *Giardia* as it has on the malarial parasite. One-sixth of a 0·1 gm. tablet given three times a day for 5 days to a child of 1 to 2 years of age usually effects a complete cure. After treatment, most children seem to remain immune for a long time, but a proportion of them become re-infected after a year or so, and may again suffer from enteritis.

Infective Enteritis, secondary to or associated with Parenteral Infection

The observation that infants and young children suffering from inflammation of the respiratory, or less often of the urinary, tract may develop diarrhœa and vomiting, has led many clinical workers to believe that practically all infective gastro-enteritis is secondary to parenteral infection. We do not propose to discuss this thesis in detail (see Smellie 1939). Careful bacteriological and pathological studies have failed to support it, and in its extreme form it is clearly untenable. Nevertheless, parenteral infection is very often present in acute gastro-enteritis, and it should invariably be sought for and excluded.

Smellie (1939) in Birmingham found that of 500 patients suffering from enteritis on admission to hospital, 231 showed unequivocal signs of parenteral infection—mainly otitis, mastoiditis or bronchitis—that 90 others developed clinical signs of parenteral infection after admission, and that 28 further children who died and came to autopsy were found to have an infection that had not been detected clinically. Bonaba and Peluffo (1940) in Uruguay recognized the presence of parenteral infection—mainly rhino-pharyngitis and otitis—in 379 out of their series of 768 patients. Campbell and Cunningham (1941), at the Park Hospital, London, studying 574 cases of non-specific gastro-enteritis, observed that 175 of them had parenteral infection on admission—mainly tonsillitis, laryngitis, bronchitis, or bronchopneumonia—and that 58 others developed it after admission. The first set had a case mortality of 23·4 per cent., the second set of 32·5 per cent. The average case mortality for the whole series of 574 cases was 27·7 per cent., so that the occurrence of parenteral infection did not appear to have much effect on the fatality of the disease.

In estimating the causal relationship of parenteral infection to gastro-enteritis it is well to remember that large numbers of children who suffer from respiratory infection do not develop gastro-enteritis, and that often quite a high proportion of cases of true dysentery develop parenteral infection (Marriott *et al.* 1933). Respiratory infections are much commoner in the winter than the summer, whereas gastro-enteritis tends, if anything, to show the reverse seasonal incidence. It is improbable, therefore, that parenteral infection is responsible for more than a proportion of cases of gastro-enteritis.

Prophylaxis and Treatment

Broadly speaking, the prevention of enteritis lies in the protection of the food supply from contamination, the provision of good general hygienic conditions, and the separation as far as possible from each other of infants and children under 2 years of age. Reference has already been made under summer diarrhœa to the beneficial effect of reducing the fly population, and to the replacement of raw by pasteurized, boiled or dried milk. Whenever possible, breast-feeding should be preferred to artificial feeding. Raw cows' milk may be contaminated from so many different sources—the cow itself, the personnel handling the milk, impure

water used for cleansing the utensils, dirty bottles, flies, and rodents—that it should never be given to infants and young children. Even milk produced under cleanly conditions on the farm, such as Tuberculin Tested milk, may be contaminated from one or more of the sources just mentioned, and should therefore always be pasteurized or brought rapidly to the boil and cooled. The importance of good hygienic conditions is obvious. In particular, care should be exercised to protect infants against exposure to undue heat or cold, and to minimize their risk of contact with other persons suffering from respiratory or intestinal infection. The desirability of rearing infants during their first two years of life under relatively segregated conditions is not yet fully appreciated. Infantile diarrhœa is so common in institutions that no encouragement should be given to the establishment of nurseries for children under 2 years of age. No infant should be admitted to a hospital for treatment of any form if this can be carried out effectively at home (see Field, MacCarthy, and Wyllie 1943). Numbers of infants die unnecessarily every year as the result of contracting gastro-enteritis in hospitals to which they have been admitted, sometimes for relatively trivial causes. Doctors and nurses should be taught to realize that the decision to admit an infant to hospital is one that should never be taken lightly; the consequences may easily be fatal. There are diseases, however, which cannot adequately be treated in the home. Infants' wards should therefore be divided into cubicles, and each occupant treated with strict precautions to avoid cross-infection. Even the maternity home should be run ideally on the cubicle system. When this is not possible, infants should be warded with their mothers and not taken away to sleep in a common nursery where, if infection is introduced, it is liable to spread rapidly.

Once gastro-enteritis has broken out in a hospital or maternity home, the measures to control it must depend to some extent on its nature and severity. Neonatal diarrhœa demands instant and rigorous action. The maternity home should be closed at once to further admissions, affected infants must be removed to suitable isolation quarters, apparently healthy infants should be sent home and kept under supervision, and the whole premises must be thoroughly cleaned and disinfected. Dysentery in a children's ward is often difficult to eradicate, but the prompt removal of all recognized cases, the detection and segregation of carriers among both the patients and the nurses, and the proper disinfection of napkins and bed linen will often prove successful.

The treatment of the individual patient falls largely outside our scope. It is interesting, however, to note that sulphonamide treatment may be of value, not only in dysenteric infections (see Chapter 70), but even in neonatal diarrhœa. Henderson (1943) has reported a striking effect of sulphaguanidine on the case mortality of this disease in the Simpson Maternity Hospital, Edinburgh. He recommends that the drug should be given in a dosage of 0·5 gm. every 4 hours for several days, and that it should not be reduced until there is clinical improvement and return of appetite. On the other hand, in ordinary infantile gastro-enteritis McSweeney (1943) observed no benefit from treatment with sulphaguanidine. Promising results have been reported by Twyman and Horton (1943) from the use of succinyl sulphathiazole.

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CHAPTER 72

BACTERIAL FOOD POISONING AND BOTULISM

FOOD POISONING

Definition.—The consumption of unwholesome food or drink may give rise not only to well-known bacterial infections such as enteric fever, dysentery, cholera, tuberculosis, undulant fever, scarlet fever, septic sore throat, and so on, and to helminth infections such as trichinosis, in which the food serves as a vehicle for the specific infective organism, but also to such diverse conditions as those due to the ingestion of inherently poisonous substances—for example deadly nightshade, hemlock, poisonous fish, toadstools, and ergot-infected grain—and those in which the food has become contaminated directly with such poisons as arsenic, tin, antimony, cadmium or other heavy metals, or with chemicals such as sodium fluoride, or tri-ortho cresyl phosphate, which was responsible for outbreaks of so-called "jake" or "ginger" paralysis in the United States (see Smith and Elvove 1931) and for a similar outbreak in Durban (Sampson 1942). The term "food poisoning," however, is conventionally restricted to acute gastro-enteritis due to the bacterial infection of food or drink. So defined, it is divided into two groups: (1) that following the multiplication within the body of pathogenic organisms contained in the food; this may be referred to as the "infection" type, (2) that following the ingestion of food in which poisonous substances have been formed as the result of bacterial proliferation; this may be referred to as the "toxin" type.

Botulism, which is also a consequence of bacterial proliferation in food, differs from either of the types just described in the character of the symptoms and in the nature of the toxin involved; though, for convenience, it will be discussed in this chapter in a separate section (p. 1612). Those interested in chemical food poisoning may be referred to the works of Jordan (1931), Tanner (1933) and Dack (1943).

It will be noted that the definition given above excludes food idiosyncrasy and food allergy—conditions in which the food itself is wholesome but the patient's reaction is abnormal. In addition, it excludes acute gastro-enteritis, such as is characteristic of the winter vomiting disease (see p. 1798), in which the infection does not seem to be food-borne; and gastro-enteritis associated with malnutrition and starvation (see García and Covián 1942).

Symptomatology.

In the *infection* type of food poisoning, after an incubation period varying from 2 to 36 hours, but generally between 8 and 24 hours, the illness commences with severe headache, followed by nausea, vomiting, diarrhœa and abdominal pain. Fever is usually present; the temperature may rise to 102° F. In favourable cases

the symptoms gradually abate so that the patient is better within a week, but in very severe cases restlessness occurs, followed by extreme thirst, cramp, coma, and death. At autopsy the mucosa of the stomach and intestines is swollen and often intensely congested. Peyer's patches are not particularly affected. Minute ulcers may be seen. Microscopic examination reveals fatty degeneration of the liver. The causative bacilli can be isolated from the heart's blood, spleen, and other viscera in a considerable proportion of fatal cases.

In the *toxin* type of food poisoning, the incubation period as a rule is much shorter—1 to 6 hours, usually 3 hours—though in some outbreaks, probably of non-staphylococcal type, it may be as long as 12 hours. The general symptoms resemble those of the infection type, but vomiting tends to be more violent, diarrhœa is less conspicuous, prostration is greater, fever may be absent, and recovery is more rapid, the patient often being well again within 24 hours. These cases are rarely fatal, so that adequate information about the pathological changes is lacking.

Epidemiology.

Incidence.—It is difficult, in the absence of compulsory notification, to estimate the frequency of food poisoning in this country. From January, 1921, to October, 1923, Savage and White (1925*b*) collected details of 86 outbreaks in Great Britain, involving about 2,300 persons with 24 deaths. But it is probable that a number of other outbreaks occurred which were not sufficiently important to attract attention. In America, Jordan calculated in 1917 that about 15,000 to 20,000 persons a year were attacked. The country that suffers most severely is Germany, probably on account of the large amount of uncooked meat that is consumed. The following figures (Table 147) refer only to outbreaks that were due to meat or meat products. The number of outbreaks from all causes was probably much higher.

TABLE 147

INCIDENCE OF FOOD POISONING IN GERMANY FOLLOWING THE CONSUMPTION OF MEAT AND MEAT PRODUCTS.

(Meyer 1929)

Year.	No. of Outbreaks.	Cases.	Deaths.
1923	76	3,746	28
1924	62	1,712	21
1925	83	1,905	22
1926	84	2,679	17
1927	110	3,548	27
1928	78	977	18

A special survey by the United States Public Health Service revealed, in 1938, 51 outbreaks of food poisoning and gastro-enteritis caused by milk or food affecting 2,459 persons (Frank 1940), and, in 1939, 142 outbreaks affecting 3,976 persons (Fuchs 1941). Reasons were advanced for believing that the total incidence was probably five, ten, or more times as great as that indicated by the collected figures. In England and Wales, the average annual number of outbreaks brought to the notice of the Ministry of Health (Scott 1939) during the years 1929–38 was 69 (see Table 148), but during the second world war, partly because of the increase in communal feeding and partly because of better reporting and investigation, this figure rose very considerably.

TABLE 148

RECORDS OF FOOD POISONING OUTBREAKS IN ENGLAND AND WALES, 1929–38.
(Modified from Scott, 1939)

Year	Total No. of Outbreaks.	*Salmonella* Outbreaks.			Dysentery Outbreaks.	Toxin Outbreaks.	Outbreaks of Doubtful Origin.
		No. of Outbreaks.	Cases.	Deaths.			
1929. .	36	9	68	4	4	5	18
1930. .	25	11	292	16	8	5	1
1931. .	36	18	425	10	2	6	10
1932. .	36*	24	186	8	10	6	?
1933. .	66	32	512	15	4	21	9
1934. .	79	43	125	6	6	24	6
1935. .	113	46	698	23	12	47	6
1936. .	69	19	224	3	8	26	16
1937. .	88	45	601	24	5	22	16
1938. .	138	72	330	8	8	21	37
Total .	686	319	3,461	117	67	183	119

* This number appears to be too low. It may also be noted that the total number of outbreaks given in this table is 69 less than the aggregate recorded in the annual reports of the Chief Medical Officer of the Ministry of Health.

Solitary cases of food poisoning, in which one member only of a family consuming similar food is attacked, are apt to escape diagnosis, the symptoms being ascribed to indigestion, chill, or some other cause, unless a bacteriological examination is made. Such cases are being detected with increasing frequency.

Season.—In this country food poisoning is commoner during the summer than the winter. Savage and White (1925*b*) found that about 72 per cent. of outbreaks occur between May and October. This is probably due to the greater rapidity with which bacteria are able to multiply under warm conditions. But the seasonal incidence depends to a certain extent on the proportion of canned to fresh food that is eaten. In America, for example, it appears to be customary for the housewife to can her vegetable produce in the summer months and to consume it during the winter; since the process of home canning is not altogether satisfactory, outbreaks of poisoning due to the consumption of this food are fairly common in the winter months. Many of these outbreaks, however, are of the botulism type.

Morbidity and Mortality.—One of the striking facts is the high morbidity rate of most outbreaks. Often all who eat the affected food are attacked. Geiger (1923) estimates it at 75–100 per cent. An exception may be made in the case of milk; Savage and White (1925*b*) state that in salmonella infections conveyed by milk the morbidity rate is only 40–50 per cent. Another striking characteristic is the low mortality rate; this is estimated by Geiger (1923) in America as less than 0·5 per cent., by Savage (1920) in England as 1·5 per cent., and by Lentz (1924) in Germany as 4·5 per cent. This last figure probably included cases of botulism, and is therefore too high. Mayer (1913) in Germany, dealing purely with salmonella outbreaks, recorded a mortality rate of only 1 per cent. Taking milk outbreaks alone, Savage and White (1925*b*) find the mortality rate to be less

than 0·2 per cent. Sex has apparently little influence. As regards age, the disease seems to be more fatal in the very young, and in the old.

General Bacteriology of Food Poisoning

Before discussing in detail the various factors concerned in the production of bacterial food poisoning, it may be well to take a brief view of the subject as a whole. Towards the end of last century, largely owing to the work of Brieger (1889), food poisoning came to be regarded as due to the presence in the food of toxic amines or *ptomaines* that had been formed as the result of protein decomposition. The work of Vaillard (1902), Fornario (1906), Cathcart (1906*b*), Savage (1921), and others, however, showed that these bodies were relatively non-toxic to laboratory animals, except when given in doses larger than were ever likely to be ingested under natural conditions. The fact, moreover, that the ptomaines did not appear till protein decomposition had reached an advanced stage, when the food would be repugnant to the normal senses, taken in conjunction with the observation that food poisoning usually results from food which is quite sound in appearance, rendered it doubtful whether these bodies played any part in the causation of the type of illness we are considering.

Belief in ptomaine poisoning received its death-blow, so far as scientific bacteriology was concerned, when it was found that in many outbreaks the food contained organisms of the *Salmonella*, or occasionally the *Shigella*, group. As the result of the work of Savage in this country, and of Jordan in the United States, food poisoning came to be regarded almost as synonymous with food-borne salmonella infection. Further work, however, failed to reveal the presence of these organisms in a considerable proportion of the outbreaks in which an adequate investigation was carried out, particularly in outbreaks following the consumption of canned food. To meet this difficulty Savage (1920, 1923, 1932) (see also Savage and White 1925*a*, *b*) put forward the attractive hypothesis that these outbreaks were due to the ingestion by the patient not of the living salmonella organisms, but of their toxins. It was supposed that the organisms had multiplied in the food prior to its consumption, and had formed thermostable toxic substances. The subsequent cooking to which the food was exposed destroyed the organisms themselves, but did not seriously affect their toxic products, which were therefore able to give rise to food poisoning on ingestion. No adequate confirmatory evidence of the formation of specific exotoxins by members of the *Salmonella* group was forthcoming, and the balance of evidence appeared to be against this view (but see p. 1607).

While bacteriologists were speculating about the causation of the non-salmonella food-poisoning outbreaks, Dack, Jordan, and their colleagues in Chicago (see p. 1605) drew attention to certain outbreaks of poisoning which were proved to be due to the presence in the food of a toxin formed by staphylococci. By observations made on human volunteers, it was shown that certain strains of staphylococci were able to form toxic substances endowed with some degree of thermostability. These findings were of special interest in that they afforded conclusive proof of the formation of bacterial toxins in the food prior to its consumption. Hitherto the only known instance of this was that of botulism—a condition to be described later, in which the anaerobic bacillus, *Cl. botulinum*, gives rise to an extremely potent specific exotoxin with an action, not on the gastro-intestinal mucosa, but on the central nervous system.

Other observations by Jordan and by various continental workers cast serious suspicion on food in which there had been inordinate bacterial multiplication of any type. The inference to be drawn from these observations was that many organisms, non-pathogenic in themselves, such as *Bact. coli*, *Proteus vulgaris*, *Proteus morgani*, milk streptococci and others, when allowed to multiply under favourable conditions in suitable types of food-stuffs, led to the appearance of toxic substances having an irritating effect on the human gastro-intestinal mucosa. Many workers had toyed with this idea of non-specific toxin formation, but had abandoned it in the absence of adequate evidence. The epidemiological, and more recently the bacteriological, evidence seems to us, however, to be becoming increasingly strong in favour of this view. The pendulum is in fact slowly swinging in the direction of the old " ptomaine " theory, with the distinction that the poisons now regarded as responsible are not the result of advanced protein decomposition, but result from the growth of types of bacteria which can proliferate enormously without greatly altering the appearance and taste of the food. What the nature of these products is remains in doubt. The suggestion that they consist of the autolysed bacterial bodies themselves (see Jordan 1931, Jordan and Burrows 1935, Savage 1933, Report 1934) is not borne out by human volunteer experiments, and it seems more probable that they consist of some breakdown product formed from the food, or of a bacterial enzyme which has a specific affinity for some constituent of the human gastro-intestinal tract.

Summing up, we may say that bacterial food poisoning may be due (1) to infection with living *Salmonella*, or occasionally dysentery, bacilli, (2) to intoxication with substances, whose nature and mode of origin is as yet imperfectly understood, which are formed as the result of undue bacterial multiplication in the food.

Bacteriology and Epidemiology of the " Infection " type of Food Poisoning

In 1888 Gaertner isolated an organism known as *Salm. enteritidis*, from the meat of an emergency-slaughtered cow, at Frankenhausen in Germany. The meat had been consumed by 58 persons, all of whom developed food poisoning. One man who had eaten about 1½ lb. died in 36 hours. At autopsy, the same bacillus was isolated from the organs of the cadaver as from the meat. In 1898, Durham in England and de Nobele in Belgium described another organism, which they had isolated simultaneously from patients suffering from meat poisoning and from the meat that they had consumed. This organism was known until recently as *Salm. œrtrycke*—Ærtrycke being the name of the Belgian village where the outbreak occurred. It is now, however, called *Salm. typhi-murium*, this being the name given to an organism which was isolated by Loeffler in 1892 from a mouse epizoötic, and which was subsequently found to be identical with *Salm. œrtrycke*.

The intensive study of the *Salmonella* group during the past few years has brought to light a number of other organisms concerned in the production of food poisoning. The frequency distribution of these types varies in different countries. Table 149 shows the distribution in Great Britain, as revealed by examinations carried out from 1923 by the late Dr. W. M. Scott at the Ministry of Health laboratory, and from 1941 onwards by the Emergency Public Health Laboratory Service.

It will be noted that up till 1942 all but a few strains belonged to 14 types, the most important of which were *Salm. typhi-murium*, *Salm. enteritidis*, *Salm. thompson*, *Salm. newport* and *Salm. choleræ-suis*. Of the remaining types listed

TABLE 149

TYPES OF *SALMONELLA* ISOLATED FROM CASES OR OUTBREAKS OF FOOD POISONING IN ENGLAND AND WALES.

	1923–39.	1940.	1941.	1942.	1943.	1944.	Total.
Salm. typhi-murium	234	45	65	35	108	185	672
Salm. enteritidis	54	18	10	11	24	55	172
Salm. thompson	49	25	20	9	19	20	142
Salm. newport	29	13	5	11	10	91	159
Salm. choleræ-suis	14	1	2	3	1	1	22
Salm. bovis-morbificans	8	3	—	3	6	3	23
Salm. dublin	8	5	1	2	2	1	19
Salm. potsdam	3	2	2	—	—	—	7
Salm. senftenberg (newcastle)	3	2	—	1	2	1	9
Salm. derby	2	4	—	—	2	3	11
Salm. eastbourne	2	—	—	—	—	—	2
Salm. stanley	3	—	1	—	—	—	4
Salm. london	1	9	—	—	—	—	10
Salm. aberdeen	3	2	1	—	—	—	6
Salm. gallinarum	—	1	—	—	—	—	1
Salm. newington	—	1	—	—	—	—	1
Salm. anatum-like	—	1	—	—	—	—	1
Salm. anatum	—	—	1	2	4	4	11
Salm. reading	—	—	3	—	—	—	3
Salm. montevideo	—	—	2	1	17	17	37
Salm. oranienburg	—	—	—	10	30	36	76
Salm. bareilly	—	—	—	1	3	2	6
Salm. braenderup	—	—	—	1	—	—	1
Salm. essen	—	—	—	1	—	—	1
Salm. oslo	—	—	—	1	—	—	1
Salm. virchow	—	—	—	1	—	—	1
Salm. meleagridis	—	—	—	1	7	11	19
Salm. chester	—	—	—	—	5	1	6
Salm. kentucky	—	—	—	—	2	1	3
Salm. javiana	—	—	—	—	1	—	1
Salm. san-diego	—	—	—	—	1	—	1
Salm. sundsvall	—	—	—	—	1	—	1
Salm. tennessee	—	—	—	—	4	2	6
Salm. muenchen	—	—	—	—	2	—	2
Salm. cardiff	—	—	—	1	—	—	1
Salm. concord	—	—	1	—	—	—	1
Salm. adelaide	—	—	—	—	4	—	4
Salm. give	—	—	—	—	—	1	1
Salm. new-brunswick	—	—	—	—	—	1	1
Salm. panama	—	—	—	—	—	1	1
Salm. pullorum	—	—	—	—	—	1	1
Salm. worthington	—	—	—	—	—	1	1
Salm. saint-paul	—	—	—	—	—	1	1
Unidentified	15	7	6	9	7	13	57
	428	139	120	104	262	453	1,506

NOTE.—The figures refer to the number of outbreaks, *not* to the number of strains studied.

above the line, all but *Salm. bovis-morbificans, Salm. potsdam* and *Salm. senftenberg* had been first isolated in Great Britain itself. In 1942, however, a remarkable change became apparent. In addition to the old well-recognized types, 10 new types were isolated, the commonest of which was *Salm. oranienburg*. In 1943,

another 8 new types were identified. With the exception of *Salm. chester* and *Salm. cardiff*, every one of these new types had been first described abroad, many of them in the United States. From epidemiological investigations made at the time there is little doubt that the majority of the new types were introduced into Great Britain by American spray-dried egg, which was first distributed on a large scale in the middle of 1942 (see p. 1599).

In the United States, Edwards and Bruner (1943) published records of 194 salmonella strains isolated from the fæces of patients suffering from gastro-enteritis. Of these, the commonest were as follows : *Salm. typhi-murium* 45, *Salm. newport* 31, *Salm. panama* 14, *Salm. oranienburg* 13, *Salm. san-diego* 11, *Salm. bareilly* 9, *Salm. montevideo* 9, *Salm. enteritidis* 8, *Salm. give* 7, *Salm. anatum* 7, *Salm. choleræ-suis* 6, *Salm. newington* 5, *Salm. bredeney* 5 and *Salm. thompson* 4. Many of these, it will be observed, were the same as the types met with from 1942 onwards in Great Britain. The comparative infrequency in the American list of *Salm. enteritidis* and *Salm. thompson*, which were the second and third commonest organisms in Great Britain before the war, is very striking. Further records of the distribution of salmonella strains in the United States will be found in papers by Seligmann, Saphra and Wassermann (1943) and Rubenstein, Feemster and Smith (1944).

In Denmark, during the years 1936–40, the types most often responsible for outbreaks of food poisoning were *Salm. typhi-murium*, *Salm. enteritidis* and *Salm. dublin* (Harhoff 1941). In all three countries, therefore, *Salm. typhi-murium* heads the list.

Besides members of the *Salmonella* group, certain organisms of the dysentery group, particularly *Sh. sonnei* and *Sh. flexneri*, may be concerned in the production of food poisoning. More usually these organisms give rise to dysentery, but occasionally their ingestion in food is followed by typical gastro-enteritis (Clayton and Hunter 1928, Sowden 1933, Scott 1936).

The proportion of total food-poisoning outbreaks constituted by salmonella infections is difficult to assess accurately. In 112 outbreaks in the British Isles recorded by Savage (1920), 39 were shown to be due to bacilli of this group. In 100 later outbreaks recorded by Savage and White (1925*b*), these bacilli were isolated in only 20 instances. Scott's experience at the Ministry of Health (see Table 148) suggests that about 40 per cent. of the severer outbreaks are due to salmonella infections.

Animal Reservoirs of Salmonella.—Though human infection with *Salmonella* is widespread and frequent, the majority of food-poisoning outbreaks due to this group of organisms follow the consumption of food directly or indirectly associated with infection of some animal. We may therefore consider briefly the distribution of *Salmonella* in the common domestic animals and birds.

Ducks.—Salmonella infection of these birds, mainly with the *typhi-murium*, *anatum*, *enteritidis* var. *essen*, *dublin* and *newington* types, appears to be not uncommon.

Beller and Reinhard (1934) in Germany, who examined 1,500 ducks' eggs from 34 farms, found that about 1 per cent. of them contained salmonella organisms. Infection was present on seven of the farms, and about 2–8 per cent. of the eggs from these farms were infected. Lerche (1936*b*) isolated *Salm. typhi-murium* from 19 out of 332 ducks' eggs bought in Berlin. Edwards (1939) refers to 5 outbreaks in the United States due to *Salmonella*. In this country Gordon (1940) examined 21,000 samples of blood from adult ducks, and found agglutinins to *Salm. typhi-murium* or *Salm. enteritidis* in 4·6 per cent. of them.

Salmonella infection of **fowls,** i.e. chickens and hens, appears to be more widespread than in ducks, though according to Gordon (1940) the resulting disease is less often fatal in chicks than in ducklings.

Infection of fowls in Germany with *Salm. typhi-murium* has been described by Beller and Zeki (1934), and with *Salm. dublin* by Lütje (1937). In the United States Edwards and Bruner (1943) have records of 862 outbreaks in fowls, omitting those due to *Salm. pullorum*, *Salm. gallinarum* and *Salm. paratyphi B*. It seems clear, however, that these authors use the word " fowl " in an extended sense, and probably include in it not only chickens and hens, but ducks, pigeons, turkeys and canaries. Mallmann, Ryff and Matthews (1942) have collected records of 17 different types of *Salmonella* infecting fowls, omitting the *pullorum*, *gallinarum*, *paratyphi A* and *paratyphi B* types. In addition, they found 3 new types themselves. Taking their records with those published by Edwards (1939), we give the following list of 24 species of *Salmonella* found in chickens and hens in the United States: *typhi-murium*, *anatum*, *enteritidis*, *oranienburg*, *bareilly*, *dublin*, *montevideo*, *kentucky*, *minnesota*, *worthington*, *derby*, *newport*, *choleræ-suis*, *amersfoort*, *muenchen*, *newington*, *senftenberg*, *new-brunswick*, *london*, *give*, *aberdeen*, *california*, *urbana* and *hvittingfoss*. In this country salmonella strains have been isolated from about 10 per cent. of over 6,000 samples of spray-dried egg powder, most of which came from the United States. The commonest types were *oranienburg*, *montevideo*, *meleagridis*, *bareilly* and *anatum*, but several others were met with. Twelve types, not mentioned in the list given above, were isolated, namely *bovis-morbificans*, *bredeney*, *cerro*, *habana*, *manhattan*, *oregon*, *potsdam*, *stanley*, *sundsvall*, *tennessee*, *california*, and *litchfield*. In Great Britain salmonella infection of fowls is not uncommon; apart from *Salm. pullorum* and *Salm. gallinarum*, the chief type isolated so far has been *Salm. thompson*.

From these results it may be concluded that, at least in the United States, infection of fowls with potential food-poisoning types is widespread, and that eggs or egg products are often infected with *Salmonella*. It is well known, of course, that fowls often suffer from bacillary white diarrhœa due to *Salm. pullorum*, and ducks from keel disease due to *Salm. anatum* ; but, though both these organisms may occasionally infect man, neither is of the same public health importance as the more common food-poisoning types.

The eggs may be infected during their formation in the oviduct, or by passage of the organisms through the shell from the fæces on the exterior (Scott 1933, Kathe and Lerche 1936, Lerche 1936*b*, Jansen 1937). Salmonella organisms may survive for at least 5 months at room temperature in infected fæces (Lerche 1936*a*). Their passage through the shell depends on a number of factors. Provided it is dry, little penetration is likely to occur, and the bactericidal effect of the egg albumen will probably prevent multiplication of the few organisms that may gain access to the interior. If, however, a warm, newly laid egg is brought into contact with fluid containing bacteria at a lower temperature than the egg itself, bacteria are readily sucked through the shell as the egg cools (Haines and Moran 1940). Whether multiplication occurs in the yolk is doubtful; but it is well to remember that, according to Prescott and Tanner (1938), most foods must be stored at 5° C. or less to ensure that no growth of these organisms takes place.

Pigs rival fowls in the frequency with which they are infected with *Salmonella*.

In Uruguay, Hormaeche and Salsamendi (1936) examined the pooled mesenteric lymph nodes from 20 normal animals at a time. Salmonella organisms were isolated from 22 out of 52 batches. An even higher proportion of positive results was obtained by the same authors (1939) in a later investigation. In this country, Scott (1940) examined pooled mesenteric lymph nodes and pooled spleens from apparently normal pigs, and

isolated *Salmonella* from 36 out of 100 batches, representing an aggregate of 1,000 pigs. The lymph nodes were far more often infected than the spleen. In the United States, Rubin, Scherago and Weaver (1942) found that about 10 per cent. of hogs in Lexington contained *Salmonella* in their mesenteric lymph nodes; and Cherry, Scherago and Weaver (1943) isolated *Salmonella* from 5·9 per cent. of retail pig products, mainly brain, chops and liver. In Mexico, Varela and Zozaya (1942) isolated 15 strains of *Salmonella* from mesenteric lymph nodes taken from a total of 209 apparently healthy pigs. The types found in these different countries comprised *choleræ-suis, typhi-murium, thompson, anatum, newport, derby, senftenberg, enteritidis, london, dublin, reading, montevideo, bredeney, newington, muenchen, chester, heidelberg, meleagridis* and *give*. In addition, Edwards and Bruner (1943) include occasional strains of *san-diego, california, bareilly, oregon, manhattan, panama, pullorum, lexington, new-brunswick, illinois, worthington, wichita* and *urbana*. *Salm. choleræ-suis* is probably the commonest type, because of the frequency with which it is found as a secondary invader in swine fever.

Cattle constitute a less important reservoir for *Salmonella* than either fowls or pigs; and the number of different types that have been isolated from them is considerably less.

The observations of Knoth (1936), Bartel (1938), Pohl (1938), and Lütje (1939) in Germany have shown that the two commonest types are *Salm. dublin* and *Salm. typhi-murium*, but that other types, met with much less frequently, include *choleræ-suis, enteritidis, rostock, abortus-equi, abortus-ovis* and *bovis-morbificans*. *Salm. dublin* is pathogenic particularly for calves, in which it gives rise to enteritis or so-called calf dysentery, with or without bronchopneumonia; the joints may be affected, and occasionally a general septicæmia occurs without localizing symptoms. *Salm. typhi-murium*, on the other hand, is found more often in adult cattle, usually in association with severe enteritis. In fatal infections both organisms can often be demonstrated in the spleen, liver, gall-bladder, and hepatic lymph nodes, as well as in the intestine. Metritis, mastitis, and nephritis are sometimes seen.

Apart from *Salm. abortus-ovis*, which may cause abortion, **sheep** are seldom infected with *Salmonella*. The *dublin, typhi-murium* and *enteritidis* types have been isolated occasionally (Bartel 1938), but infection with any of these organisms appears to be rare.

From **horses** the *dublin, typhi-murium, enteritidis* and *bovis-morbificans* types have been isolated (Bartel 1938), as well as the more frequent *Salm. abortus-equi*. The incidence of salmonella infection in these animals is still imperfectly known.

Rodents.—Both rats and mice suffer naturally from infection with *Salm. typhi-murium* and *Salm. enteritidis*.

Savage and White (1923) examined 96 rats from two slaughter-houses. From 6 of them—all from one slaughter-house—*Salm. enteritidis* was isolated; 3 of them were harbouring the organism in their intestines and had presumably been excreting it before death. Moreover, 20 out of 66 rats examined contained agglutinins in their blood serum for *Salm. enteritidis*, varying in titre from 1/40 to 1/800 or higher, probably indicative of either an old or a recent infection. They quote the findings of Savage and Read, who isolated this organism from 5 out of 41 rats examined in 1913. More recently Khalil (1938) examined 750 wild rats trapped in Liverpool. About 70 per cent. were of the large brown and 30 per cent. of the small black variety. Cultures were put up from the liver, spleen and gut. *Salmonella* was isolated from 55 rats, i.e. 7·3 per cent. Of a total of 89 strains isolated, 45 belonged to the *enteritidis*, 40 to the *typhi-murium*, 3 to the *newport*, and 1 to the *thompson* type. In the United States, Welch, Ostrolenk and Bartram (1941) report the isolation of *Salmonella* from 5 out of 420 samples of rat and mouse fæces received from different parts of the country, i.e. 1·2 per cent. The studies

of Topley and his colleagues in this country and of Webster and his colleagues in the United States (see Chapter 56) have shown that infection of rodents with *Salmonella* may result in an acute rapidly fatal septicæmia, a subacute enteritis which may or may not prove fatal, a chronic disease accompanied by changes in the liver and spleen, or by a completely symptomless infection.

Type of Food.—In salmonella outbreaks the incriminated food has generally been meat, milk, fish, or other animal protein such as eggs; vegetables and cereals are less commonly, and fruits rarely, implicated. Moreover the meat has often been made up into pâtés, pies, brawn, jellies, sausages, or other similar products—a process that involves imperfect cooking and liability to contamination. Fresh meat that has been responsible for poisoning will on investigation generally be found to have been stuffed, or re-cooked, or otherwise treated; or it may prove to be ham that has been imperfectly pickled or cooked.

In some outbreaks the food has been prepared and allowed to stand for a time before being eaten; in others it has been heated up again on the 2nd or 3rd days; in others, especially in hot weather, it has been placed in the oven for an hour or so "to prevent its going bad," and not cooked and eaten till the following day.

It is important to remember that the food may, and generally does, appear quite normal to ordinary inspection. To illustrate this, van Ermengem (1896) tells a graphic story of a meat inspector in Belgium who was asked to give his opinion on some suspected sausages. Judging from their excellent appearance, their good smell, and their rosy colour, he pronounced them suitable for consumption, and to demonstrate their harmlessness he ate three slices himself. He developed acute gastro-enteritis, and died in 5 days. From his viscera at autopsy, and from the sausages, *Salm. enteritidis* was isolated. This story illustrates another point of importance—namely, that the distribution of bacilli in an article of food may be quite uneven. Though a large number of sausages were made from the same meat on the same day, only four of them were infected. This is a frequent observation, particularly in relation to canned foods; only one or two cans of a large batch may prove harmful, showing that either the original contamination was localized, or that in the process of sterilization one or two cans, through bad packing or some other cause, failed to be raised to the temperature required to kill the bacilli.

In 68·8 per cent. of the 112 British outbreaks recorded by Savage (1920), in which animal food was the vehicle, the meat was derived from the pig or the ox. Experience in other countries has been much the same. Meyer (1929), for example, reports that, in Germany between the years 1923 and 1928, out of a total of 281 outbreaks in which the animal source of origin was determined, 168 were due to cattle, 60 to pig, and 49 to horse flesh. In 137 other outbreaks sausage meat was the cause, and in a further 75 outbreaks meat of various types had been used.

Of recent years considerable attention has been directed towards poisoning by *eggs*. These have been derived most frequently from ducks, less often from hens and pigeons (Clarenburg and Dornickx 1932, Lovell 1932, Scott 1933, Seligmann 1935, Hohn and Herrmann 1935, Jansen 1936).

The frequency of food poisoning from this source is peculiarly difficult to ascertain, because unless the infected egg is used for preparing a custard, cream filling, or some similar dish, infection is restricted to individuals, and so does not usually come to the notice of the public health authorities. Moreover, the causal relationship is often never even suspected. During the second world war

several new types of *Salmonella* were isolated from cases of food poisoning (see Table 149, p. 1597). Circumstantial evidence suggested very strongly that most of these were derived from the consumption of imported spray-dried egg. This product was not distributed on a large scale till the middle of 1942. Bacteriological examination revealed the presence of *Salmonella* in over 10 per cent. of the samples, the most common types being the same as those most frequently isolated from cases of food poisoning, namely *oranienburg*, *montevideo*, *meleagridis*, *bareilly* and *anatum*. The close parallelism, both chronologically and qualitatively, left little doubt that the egg powder was mainly responsible. The reason why food poisoning due to this cause was not commoner than it was must be ascribed mainly to the consumption of the egg powder soon after reconstitution, so that growth had no time to occur, and to the destructive effect of cooking.

Mode of Infection of the Food.—Briefly, the meat may come from an animal infected during life with a specific organism, or it may come from a healthy animal and be contaminated during the course of preparation for eating.

Enough has already been said to show that salmonella infection of domestic animals is by no means infrequent, particularly among fowls, ducks, pigs and cattle. The question at issue is: How often does meat responsible for food poisoning come from obviously diseased or from apparently healthy animals? That *Salmonella* may give rise to acute disease in domestic animals is abundantly clear. Elkeles (1930), who has reviewed the subject, quotes figures from Standfusz which show that, of clinically recognizable infections, 22–47 per cent. are characterized by gastro-intestinal symptoms, 11–21 per cent. by septicæmia, 11–19 per cent. by disturbances occurring at parturition, and 19–48 per cent. by other manifestations. It seems probable that meat from such animals is far more likely to be dangerous to human beings than meat from animals which are suffering from no more than a latent infection of the glands or spleen. This is in accordance with epidemiological evidence, which shows that in most instances of food poisoning due to the consumption of flesh from an infected animal, the animal has been "emergency-slaughtered." In Germany, such animals afford a frequent source of human infection. No fewer than 120 of the German outbreaks recorded by Meyer (1929) between the years 1923 and 1928 were due to this cause. In Great Britain and the United States, on the other hand, the consumption of meat from obviously sick animals is far less common, and most salmonella food-poisoning outbreaks are due to contamination of meat from a healthy animal after slaughter. Exceptions do, however, occur. Animals that appear to be healthy in the abattoir may nevertheless be suffering from a generalized salmonella infection. Scott (see Report 1936), for example, records an outbreak traced to the carcase of an animal that was almost certainly suffering from septicæmia. The carcase was not noticeably unhealthy, yet from all parts of it *Salm. typhi-murium* was isolated. Ketz (1937) likewise describes an outbreak due to the same organism which was traced to the meat of a young cow. This animal had been inspected by the veterinarian before slaughter and had been reported as in prime condition. No evidence of disease was found on examination of the carcase, but *Salm. typhi-murium* was isolated from a number of animals on the farm from which the cow came. Healthy carriers of this sort are probably uncommon, but it is well to remember that neither they nor the very early septicæmic case can be detected by veterinary inspection. A further exception must be made for certain milk-borne outbreaks in which, either the udder of the cow is shown to be infected, or the milk becomes contaminated

from the infected fæces of the animal. Outbreaks of the former type have been recorded by Kinloch, Smith and Taylor (1926) and Tulloch (1939), and of the latter type by McAllan and Howie (1931) and Conybeare and Thornton (1938).

Passing on to the second method of infection, we may note that opportunities for sound meat to be contaminated during the course of preparation for food are numerous, though it is notoriously difficult to detect the way in which any given article has been infected. The main sources are rats and mice, and human carriers.

The frequency of rodent typhoid varies in different countries, and in different parts of the same country, and is often increased locally by the use of " virus " preparations containing living salmonella organisms for the extermination of rats and mice. It is well known that infected animals, while suffering from a chronic disease, or showing no obvious symptoms of illness, may excrete the causative organism in the fæces for weeks or months (Topley and Ayrton 1924). Moreover, in the fæces themselves, *Salmonella* may survive under favourable conditions for 5 months or longer (Welch *et al.* 1941). Food prepared in unsanitary premises is liable to be contaminated from the droppings of these animals. It is usually difficult to obtain conclusive evidence that the food has been infected in this way, but outbreaks suggestive of a rodent source of infection have been described by Bainbridge (1912), Willführ and Wendtlandt (1921), Spray (1926) and Jones and Wright (1936). In addition, several outbreaks are on record in which infection has been traced, directly or indirectly, to the use of " virus " preparations (see Shibayama 1907, Jordan 1931, Kristensen and Bojlén 1931, Harhoff 1941, Leslie 1942).

The part played by the *human carrier* is difficult to define. In 121 outbreaks of food poisoning investigated by Savage (1932) there were only 5 in which a human carrier seemed to have been responsible, and Savage would therefore ascribe little importance to this mode of infection. Other workers, however, such as Jordan (1917) and Geiger (1923) take a more serious view. Rubenstein, Feemster and Smith (1944) ascribed no fewer than 6 out of 17 food-poisoning outbreaks to infection by human contact. There are now numerous instances on record in which *Salmonella* has been isolated from convalescents, ambulant cases, chronic carriers, and healthy persons.

Thus Fletcher (see Perry 1920) found *Salm. typhi-murium* in the fæces of three soldiers who were convalescing from dysentery or enteric fever. Trommsdorf, Rajchman and Porter (1911), in their investigations of the Wrexham outbreak of 1910 due to pork pies, found that the head cook was a chronic carrier, and was excreting an organism of the *Salmonella* group in her fæces. Sporadic cases of infection with organisms of the *Salmonella* group are not uncommon (Rosher and Wilson 1921). A large outbreak of food poisoning in France during the war of 1914–18 due to *Salm. newport* was ascribed by Perry and Tidy (1919) to a human carrier in the cook-house.

Borman, Wheeler, West and Mickle (1943) encountered one carrier of *Salm. bareilly* and another of *Salm. worthington*—both of over a year's standing. Moreover, now that routine examinations of canteen staffs are common practice, and methods of isolation have been improved, far more salmonella carriers are being detected than formerly. Thus Borman and his colleagues found two patients at operation to be carrying *Salm. montevideo* in the gall-bladder. Bornstein, Saphra and Strauss (1941) met with healthy carriers of the *oranienburg, derby, typhi-murium, chester* and *montevideo* types—three of them in food handlers ; and Bornstein and Saphra (1942) reported the finding of healthy carriers of the *urbana* and *senftenberg* types. Four chronic carriers were met with by Rubenstein, Feemster and Smith (1944). Patients who have suffered from salmonella infection may

continue to excrete the causative organism for several weeks. Perry and Tidy (1919), for example, reporting on an outbreak in France affecting over 1,000 men, due to *Salm. typhi-murium*, found that 50 per cent. of the patients ceased to excrete the organisms by the end of the 4th week; one patient, however, was still excreting them after 14 weeks. Mosher, Wheeler, Chant and Hardy (1941) report that in another outbreak, due to *Salm. typhi-murium*, about 50 per cent. of the convalescents excreted the organism in the fæces for 2 weeks, 20 per cent. for 4 weeks, 10 per cent. for 7 weeks, and occasional patients for even longer, one continuing for 18 weeks. The experience of the Emergency Public Health Laboratory Service in England and Wales during the second world war amply confirmed these findings, and several patients were encountered who had been excreting *Salmonella* in the fæces for many months. Numerous workers, it may be noted, have found *Salmonella* in the *tonsils*. Varela and Olarte (1942), for example, in Mexico, isolated organisms of this group belonging to 8 different species from 8 per cent. of hypertrophied tonsils removed from children.

Temporary carriers and ambulant cases must always be a menace to the human population. Their detection by bacteriological methods is bound to be difficult, since their infection will often have cleared up before suspicion is cast upon them. How far the chronic human carrier is responsible for infection of food must, however, await further observation.

It may be noted that, in food-poisoning outbreaks due to food derived from a sick animal, *Salm. enteritidis* and *Salm. dublin* are most frequently found, whereas in outbreaks due to sound meat contaminated during its course of preparation for food, *Salm. typhi-murium* is the predominant organism (see Bainbridge 1912, Mayer 1913, Jordan 1917).

Before leaving this subject attention should be drawn to the apparent infrequency of salmonella food poisoning in man in spite of the widespread incidence of infection in domestic animals, and their products used for food, and in rodents. Scott (Report 1929) explained this discrepancy on the assumption that large doses of *Salmonella* were probably required for infection of man. In typhoid, cholera and probably dysentery, minute amounts of infective material may suffice to cause disease; but in food poisoning large doses are generally necessary. Savage (1942*a*) has ably developed this theme in relation to paratyphoid fever; and Hormaeche, Peluffo and Aleppo (1936) have supported it by human volunteer experiments with *Salm. typhi-murium*. These workers found that adults might ingest as many as 2,000 to 4,000 million organisms without suffering from any symptoms at all, or from not more than mild afebrile diarrhœa. Infants and old persons, however, appear to be considerably more susceptible, and fatal cases are restricted mainly to these two age groups.

Bacteriology and Epidemiology of the Toxin Type of Food Poisoning

The clinical manifestations of this type of food poisoning, which come on usually after a very short incubation period, have already been described. The type of food concerned varies considerably. Canned or potted meat or fish, meat pies, gravy, pickled or pressed beef, ham, tongue, and cream or custard fillings are among the commonest articles, but cheese, ice-cream and milk are occasionally incriminated. Numerous outbreaks in this country have followed the consumption of imported American spray-dried egg.

Our knowledge of this form of food poisoning is still fragmentary, mainly owing to the absence of any laboratory test for demonstrating toxin in the food or in bacterial cultures prepared from it. The cause of one form, however, is

now established without any reasonable doubt, namely that due to staphylococcal enterotoxin.

Staphylococcal Toxin Poisoning.—As Dack (1943) points out, the ability of some strains of staphylococci to produce a toxic substance capable of irritating the gastro-intestinal tract has been discovered four times—by Denys (1894) in Belgium, by Owen (1907) in the United States, by Barber (1914) in the Philippines, and by Dack, Cary, Woolpert and Wiggers (1930) in the United States. In 1914 Barber, working in the Philippines, reported a very clear instance of a "toxin" outbreak due to the growth of a white staphylococcus in the milk of a certain cow. Immediately after withdrawal from the udder, the milk could be consumed with impunity, but if it was allowed to stand for a few hours at room temperature, 28°–30° C., it gave rise within about 2 hours to nausea, vomiting, abdominal pain, diarrhœa, cramps, and faintness. Similar symptoms were produced in human volunteers by the consumption of pure milk cultures of the strain of *Staph. albus* isolated from the cow in question, though kittens, puppies, and monkeys proved refractory. This report furnished in its general outlines an almost complete picture of the causative factors responsible for a "toxin" outbreak. Unfortunately, however, no particular attention seems to have been paid to it, and it was not till the rediscovery several years later by Dack and his colleagues (1930) of staphylococcal food poisoning that the subject attracted the attention it deserved. Since that time numerous outbreaks caused by the production of a toxic substance elaborated by staphylococci in the food have been described (Dack *et al.* 1930, 1935, Jordan 1931, Jordan and Hall 1931, McBurney 1933, Jordan and Burrows 1934, Report 1934). Most of the outbreaks have followed the consumption of milk products, particularly cakes and éclairs filled with cream or custard, though some have been traced to gravy or to canned meat or fish. The circumstances have invariably been such as to favour the multiplication of the organisms in the food prior to its consumption.

The origin of the staphylococci is still in doubt, but in view of the frequency of these organisms in the human nasopharynx, on the human skin, and in cows' milk, contamination of food must be relatively common. Only, however, when conditions favour their growth in the food, is their presence likely to be of danger. What proportion of strains are toxigenic is not yet known. Hope of settling this point and of learning something about the conditions that favour the formation of enterotoxin was awakened by the introduction by Dolman, Wilson and Cockcroft (1936) in Canada of the intraperitoneal kitten test.

These workers found that, if a suitable strain of staphylococcus was grown in soft nutrient agar for 3 days at 37° C. in an atmosphere containing 10 per cent. CO_2, an enterotoxin was produced which, when extracted by saline and inoculated intraperitoneally into a kitten, gave rise to vomiting within about 2 hours. To avoid confusion, any hæmolysin present had to be inactivated by neutralization with specific serum, by formolization, or by boiling for 20 minutes at pH 7. Unfortunately, this method has not withstood the test of experience. The observations of Kojima and Kodama (1939), Slanetz (1942), and Fulton (1943) strongly suggest that the substance responsible in the boiled extract for causing vomiting is residual β-toxin. Fulton, for instance, was able to obtain a positive kitten response with as little as 2 μg. of a purified β-lysin. On the other hand, the β-lysin is apparently incapable of causing gastro-intestinal irritation in man (Fulton 1943, Dolman 1943). This may explain why extracts of staphylococcal cultures from some toxin outbreaks have given a negative kitten test, and why extracts of other

strains from non-epidemic sources have caused vomiting in kittens but have proved innocuous to human volunteers. Whether it is possible, as Dolman (1943) maintains, to distinguish the reaction of the kitten to enterotoxin from that due to β-lysin is doubtful; further work will be required to settle this point. Several other animal tests for detecting enterotoxin have been described (see p. 617), but none of these has so far been entirely satisfactory. In 1935 Stritar and Jordan concluded that no method existed, apart from the feeding of broth filtrates to human volunteers, of determining whether a given strain was toxigenic. With this conclusion we are still forced to agree.

Little is yet known about the nature of the toxin produced. Dolman has always insisted that it is distinct from either the α or the β-hæmolysin; but though it is now fairly well established that it cannot be identified with the β-lysin, its lack of relation to the α-lysin is not so clear. Fulton (1943) was unable to separate the two, and was of the opinion that some, though not all, α-lysins might be enterotoxic.

Reference has already been made to the apparently curious behaviour of the enterotoxin towards heat. The evidence suggests that, in common with the α and β-staphylococcal lysins, the lecithinase of *Cl. welchii* cultures, and probably certain other substances of enzymic nature, the enterotoxin may be inactivated by heating at 60° C. for 30 minutes, but may not be completely destroyed by boiling (see Fulton 1943). Confusion has arisen in the past owing to failure to realize this, and to the statement frequently made that the α-lysin is destroyed at 60° C., whereas the enterotoxin withstands heating to 100° C. (see pp. 615–617).

The enterotoxin gives rise to acute gastro-enteritis with severe collapse when consumed by human volunteers in doses of 2–10 ml., but different persons vary in their susceptibility towards it. It is relatively non-toxic when fed to most laboratory animals, though young *rhesus* monkeys may be affected after a dose of 25–50 ml. It is of doubtful antigenicity, and little tolerance to it seems to be developed in the human subject as the result of repeated dosage (see Jordan 1930, Jordan, Dack and Woolpert 1931, Dack, Jordan and Woolpert 1931*a*, Dack *et al.* 1931*b*, Jordan and Burrows 1933); though Dolman (1944) claims to have been able to immunize human subjects against it by repeated subcutaneous injections of a formol toxoid preparation.

Non-staphylococcal Toxin Poisoning.—The occurrence of staphylococcal food-poisoning outbreaks has suggested an explanation for many of the other "toxin" outbreaks. For several years banal organisms such as *Proteus vulgaris* and *Bact. coli* have been found in suspected food, but their importance has generally been discounted on the basis of their non-pathogenicity to laboratory animals. It is now becoming increasingly probable, both on epidemiological and bacteriological grounds, that a number of organisms, many of them non-pathogenic, when allowed to grow in food under suitable conditions, are able to give rise to toxic substances having a highly irritating effect on the human gastro-intestinal mucosa.

Numerous outbreaks have now been described in which no living *Salmonella* or *Shigella* could be demonstrated, but in which the incriminated food contained large numbers of organisms belonging to the *Proteus*, coliform, *Streptococcus*, or aerobic spore-bearing groups (see, for example, Glücksmann 1899, Silberschmidt 1899, Pfuhl 1900, Aoki and Sakai 1926, Linden, Turner and Thom 1926, Demnitz 1926, Jansen and Dooren de Jong 1930, Cary, Dack and Myers 1931, Gustafson 1932, Tanner and Ramsey 1932, Jordan and Burrows 1934, Gray 1937, Cary, Dack and Davison 1938, Kathe 1937, Scott 1939, Lodenkämper 1940, Cooper,

Davies and Wiseman 1941, Getting, Rubenstein and Foley 1944). Dolman (1943) is of the opinion that many, if not all, outbreaks of this type are really due to staphylococcal enterotoxin, formed by staphylococci that have subsequently died out or been overgrown by other organisms. Such a unitarian hypothesis, however attractive, is not altogether satisfactory. We have ourselves examined a number of foods from toxin outbreaks in which we have been unable, even by the use of enrichment and selective media, to demonstrate a single staphylococcus, or to detect the presence of pre-formed staphylococcal hæmolysin in an extract of the food. It is not easy to explain why a hardy organism like *Staph. aureus* should die out completely, when less resistant organisms like green-producing streptococci are present in abundance. Again, in some of the toxin outbreaks, the incubation period has been as long as 12 hours; in known staphylococcal outbreaks, on the other hand, it is usually 1–4 hours, and rarely, if ever, longer than 6 hours.

Whether the other organisms, to which we have referred, are able to form a true enterotoxin, whether they produce toxic breakdown products in the medium, or whether they act in some other way is still unknown. The observations of Cary and his collaborators (1931, 1938) suggest that green-producing streptococci may give rise to food poisoning when they are ingested in large numbers *alive*, but that they do not produce filtrable toxic substances *in vitro*. If this is true, it is difficult to understand why, in some of the streptococcal outbreaks that have been described, the incubation period has been as short as 4 hours, for little toxin production by living bacilli could be expected in so short a time.

Experimental work on the non-staphylococcal types of food-poisoning has not been fruitful.

Jordan and Burrows (1935) reported that freshly isolated strains of organisms such as streptococci, *Proteus*, *Bact. coli* and *Bact. ærogenes*, when grown under suitable conditions, were capable of producing substances that caused vomiting and other symptoms of gastro-intestinal irritation in monkeys. The exact conditions necessary to the production of these substances were not defined, but growth in a custard medium, or in a medium to which starch had been added, appeared to be particularly favourable. Hunter and Dack (1938) were unable to confirm these findings; and Dolman (1943) has likewise failed to produce vomiting in human volunteers fed with culture filtrates of strains of *Proteus vulgaris*, *Bact. coli*, streptococci and aerobic spore-bearing bacilli that had been isolated some months previously from suspected food. Negative results of this sort are always difficult to interpret, and it may well be that only some strains are toxigenic or toxigenic only under certain conditions. The question is still open, and calls for further investigation.

Whether organisms of the *Salmonella* group can produce enterotoxic substances is likewise open to dispute. There is plenty of evidence to show that they contain in their bodies thermostable, water-soluble substances which are toxic on parenteral inoculation into animals (Brieger and Fraenkel 1890, Cathcart 1906*a*, Ecker 1917, Savage and White 1925*a*, Menten 1926, Bahr and Dyssegaard 1927, Meyn 1930). Methods for extracting these bodies have been described by Boivin and Mesrobeanu, Raistrick and Topley, and Morgan (see Chapter 44), who have shown that they are of polysaccharide nature. Injected intravenously into rabbits, they give rise to great weakness and prostration, often accompanied by tremors, diarrhœa, and hyperglycæmia (Delafield 1934); and injected intraperitoneally into mice in a dose of about 0·5 mgm., they prove fatal (Martin 1934). But there is as yet no conclusive evidence that they are able to cause gastro-enteritis in man when taken by the mouth. Feeding experiments carried out on human volunteers and on

monkeys by Dack, Harmon and Jarra (1928), Dack, Cary and Harmon (1928), and Verder and Sutton (1933), using heat-killed organisms or culture filtrates of *Salm. typhi-murium* and *Salm. enteritidis*, proved uniformly negative. Moreover, the ingestion by students of even 200 mgm. of the polysaccharide fraction isolated by Raistrick and Topley (1934) from *Salm. typhi-murium* was without any obvious effect (Colbeck 1942).

Summarizing, we may say that many of the " toxin " outbreaks of food poisoning appear to be due to the production of toxic substances in the food prior to its consumption. That some strains of staphylococci are capable of forming a potent enterotoxin, there is no doubt. Whether other bacteria, particularly streptococci, coliform bacilli, *Proteus*, and aerobic spore-bearers, are able under favourable conditions to render food toxic is still undecided. There is ample scope for investigation along these lines.

The frequency of the toxin type of outbreak varies in different countries. Table 148 (p. 1594), which shows the figures for England and Wales, almost certainly underestimates, and underestimates grossly, the real incidence of the disease. The attack, though often violent, is usually over so soon, and is limited so frequently to a few persons that the health authorities get to hear of only a small proportion of the total cases. Scott (1936) estimates that probably about one-third of the reported outbreaks of food poisoning of the toxin type are due to staphylococcal intoxication, but our information is as yet too scanty to justify us in making any definite statement. One factor that greatly hinders exact knowledge on this subject is the absence of any simple laboratory method for detecting the presence of toxic substances in the food or in filtrates of broth cultures of suspected organisms. For this reason many bacteriological reports are completely negative, and no further attention is paid to the outbreaks. One fact does seem clear, namely that poisoning due to canned meat and fish is becoming much less common in this country, owing apparently to the improvements in processing made during recent years (Savage 1939). Another interesting fact is the comparative infrequency now of summer diarrhœa of infants. Though this condition is usually of an infectious nature, there is a good deal of evidence to suggest that milk in which inordinate bacterial growth has been allowed to occur may contain substances having an irritating effect on the gastro-intestinal mucosa, particularly of infants. Attention was drawn to this possibility many years ago by Park and Holt (1903). There is reason to believe that one factor in the diminution of summer diarrhœa of late years has been the much greater care exercised in the production of milk, and the frequency with which it is now submitted to pasteurization or other forms of heat treatment.

Diagnosis and Investigation of Outbreaks.

The diagnosis of food poisoning is primarily clinical. For the full investigation of an outbreak the reader is referred to textbooks of Hygiene, but the general procedure is briefly as follows: (1) Secure a complete list of cases. (2) Obtain particulars of individual cases. (3) Ascertain the vehicle of spread. (4) Determine the causal agent—chemical irritant, *Salmonella*, *Shigella*, *Cl. botulinum*, staphylococcal toxin, or other form of toxin or infection. (5) Find out how the vehicle became infected. (6) Endeavour to trace the reservoir from which the vehicle became infected, such as rodents, cattle, fowls, ducks, or human carriers (see Savage 1942*b*).

Materials collected for examination should include (1) the actual food consumed ; it is most important that this should be obtained : (2) the vomit and fæces of patients, and the blood for agglutination : (3) the blood, spleen, liver and intestine of fatal cases : (4) the fæces and blood of suspected carriers who may have contaminated the food. The material should be sent to the laboratory packed in ice. A bacteriological examination should first be made, and if this is negative, search should be made for chemical poisons or preservatives in the food.

In salmonella outbreaks the organisms can frequently be demonstrated in the food, and in the fæces of the patient ; vomited matter is much less satisfactory. In their isolation the general plan should be to plate suspensions of the suspected material on to suitable selective and differential media (see Chapter 69 and Felsenfeld 1944), both directly and after incubation in selenite or tetrathionate broth, to pick off likely colonies, to identify them roughly by the slide agglutination method using special group sera, and to complete their identification later by cultural, biochemical, and more detailed serological methods.

For a description of the general mode of investigating food-poisoning outbreaks and of identifying salmonella types, reference should be made to a memorandum by the Ministry of Health (Memo 1935), to Kauffmann's (1941) monograph, and to a paper by Edwards and Bruner (1942) ; and for rapid methods of detecting certain chemical poisons to a paper by Korff and Kaplan (1942). The occurrence of uncommon types of *Salmonella* often affords a strong indication of the probable vehicle or reservoir of infection ; and even with some of the commoner types, such as *Salm. typhi-murium* or *Salm. enteritidis*, antigenic or biochemical peculiarities may be of help in this respect. For example, absence of the V somatic antigen from *Salm. typhi-murium* suggests a pigeon source (Edwards and Bruner 1940), and the biochemical characters of the *danysz* sub-type of *Salm. enteritidis* are indicative of a rodent origin. Even more promising is the application of the bacteriophage method of typing. Felix and Callow (1943) have already been able to distinguish two types of *Salm. typhi-murium* by this means, and it is hoped that further progress along these lines will be made. The length of time during which *Salmonella* can be isolated varies considerably. Reference to p. 1604 will show that about 50 per cent. of patients continue to excrete the causative organism for 2–4 weeks. It is therefore always worth while attempting to make a retrospective diagnosis by examination of the fæces of convalescents. It must be remembered that occasional outbreaks of suspected food poisoning are caused by members of the dysentery group—especially *Sh. flexneri* and *Sh. sonnei*.

Suspected material may be inoculated into mice or guinea-pigs, but this procedure is liable to cause confusion. Laboratory rodents often suffer from natural infection with the very types of *Salmonella* that are most likely to be found in cases of food poisoning. In consequence, bacilli may be isolated from the tissues other than those contained in the inoculum. Many careful workers have fallen victim to this type of occurrence (Holth 1909, Mühlens *et al.* 1909, Zwick and Weichel 1910). A similar technique applied to the fæces of healthy animals such as cattle and pigs has also been responsible, it may be noted, for grossly misleading reports on the frequency of salmonella bacilli in normal excreta. Unless therefore a stock of animals is available which is known to be free from this type of infection, it is advisable not to use the animal inoculation method.

An indirect method, which may sometimes be of help in diagnosis, is afforded by the use of the agglutination test on the sera of the patients. The value of this

method, however, is seriously diminished by the fact (1) that the agglutinin response in infected persons is usually poor and is often absent, and (2) that salmonella agglutinins may be present in the sera of healthy persons (see Chapter 69). In patients who have suffered from enteric fever or from previous salmonella infection, or who have been inoculated with T.A.B.C. vaccine, residual agglutinins may be present; and a rise in titre within ten days or a fortnight of the food-poisoning attack may be due to an anamnestic reaction. Even if agglutinins are found in fairly high titre, they can afford no more than an indication of the antigenic group to which the infecting organism belongs. In practice it is usually sufficient to test the serum of the patients against the species of *Salmonella* that are known to be commonest in the region, choosing preferably chloroformed suspensions giving both H and O agglutination (Scott 1936; see also Bridges and Taylor 1944). Of more value epidemiologically is the testing of the serum against the infecting organism itself, if this has been isolated from one or more of the patients. In this way useful information may sometimes be obtained about the possible connexion of suspected cases or carriers with the main outbreak. But again, a negative result is of little value, since so many patients fail to develop agglutinins.

In the absence of demonstrable salmonella or dysentery infection, or of symptoms pointing to chemical poisoning, an endeavour should be made, particularly in outbreaks of the toxin type, to obtain evidence of the presence of toxic substances of bacterial origin in the food or of organisms capable of forming such substances. The investigation of this type of outbreak is remarkably difficult. Though it may be worth while feeding the suspected material to *rhesus* monkeys, the only satisfactory way is to make tests on human volunteers. Search should be made in the food for organisms such as staphylococci, streptococci, coliform, *Proteus* and aerobic spore-bearing bacilli, which may be potentially toxigenic; but little attention should as a rule be paid to any of these organisms unless it is present in large numbers. Similar organisms should be sought for in the patient's excreta. In staphylococcal outbreaks, the organisms may be found in the vomit and occasionally in the fæces; if so, they should be examined serologically or by the bacteriophage typing method (see Wilson and Atkinson 1945) to see whether they resemble the strain isolated from the food. Any organism on which serious suspicion is cast should be grown in soft agar for 2–3 days in 10–30 per cent. CO_2, and a saline extract of the culture, filtered or centrifuged to remove living organisms, fed in 2–5 ml. quantities to human volunteers.

In many of these outbreaks even a careful review of all the evidence, epidemiological and bacteriological, will justify no more than a cautious expression of opinion as to their mode of origin. As has already been pointed out, there is ample scope for investigation of this type of outbreak, and it is to be hoped that in the future an attempt will be made to ascertain the chemical nature of the bacterial toxins found in the food.

The examination of canned foods should be conducted on the same principles as that of fresh food, but there are a number of technical details to be attended to; for a description of these the reader is referred to articles by Esty and Stevenson (1925), Savage (1923), Esty (1935), Woodcock and Lewis (1938), Jones and Jones (1941), Report (1941), and Baumgartner (1943).

Prophylaxis.

The hygienic precautions necessary to prevent food poisoning concern the whole course of the food from the slaughter of the animal to the final preparation

for consumption. A thorough system of meat inspection is essential. The meat of animals that are ill or are emergency-slaughtered should as a rule be condemned. To this precaution alone Meyer (1916) attributes the comparative infrequency of meat poisoning in California, where it is known that calves are infected with *Salm. enteritidis*. The laxity of the meat inspection in Germany since the war of 1914–18 appears to have resulted in a great increase in the number of poisoning outbreaks. The measures to be taken to ensure the cleanliness of premises where made-up meat is prepared are dealt with fully in textbooks of Hygiene. Valuable control measures, affecting both equipment and personnel, are suggested in the Ordinance and Code of the United States Public Health Service regulating eating and drinking establishments (Report 1943). It is particularly important not to employ cooks having a history of previous enteric infection. Any food-handler suffering from vomiting or diarrhœa should be suspended from work or given other occupation. Everything should be done to prevent the access of rats, mice, flies (see Ostrolenk and Welch 1942), and other animals or insects to food destined for human consumption.

Meat should be used fresh whenever possible, and should be thoroughly cooked before it is eaten. Cooking, it must be understood, does not necessarily sterilize the meat. The penetration of heat into a large joint is slow, and the interior may not reach, or may not be maintained long enough at, a temperature sufficient to kill all the organisms. This is especially true of food contained in cans, which, even after heating to above the boiling-point for an hour or more, may yet contain living organisms—generally thermophilic or spore-bearing bacilli or resistant micrococci. Several workers have shown that about 70 per cent. of samples of canned foods are not sterile (Vaillard 1902, Bushnell 1922, Savage 1923). On the other hand, food poisoning is far less often caused by canned foods than by made-up foods. This is to be attributed partly to the greater heating that the former receive, but largely to the much greater care that is used in their preparation. But cooking is a wise precautionary measure; even if it fails to kill all organisms, it probably succeeds in diminishing their numbers. If the food is not to be eaten immediately after it has been cooked, it should be placed in a cool situation, preferably in an ice-chest; otherwise organisms that have escaped destruction may multiply abundantly. That this is true is manifested by the Chesterfield outbreak, which was due to the consumption of pork pies. In persons who ate them 2 days after they had been cooked, the incubation period was 22 hours; in those who ate them 4 days after cooking it was 20 hours; 6 days after cooking it was 14 hours, and 7 days after it was only $2\frac{1}{4}$ hours, suggesting that the organisms had multiplied abundantly since the preparation of the pies (see also Baars 1931). This precaution applies to all foods, especially milk. After pasteurization or boiling the milk should be rapidly cooled. In the preparation of ice-cream Buchan (1910) found that an enormous increase of bacteria occurred during the process of slow cooling that is employed after the preliminary heating.

The protection of custard and cream fillings and of made-up meat dishes, such as brawn and pressed beef, from staphylococcal infection of human origin requires the greatest care and vigilance. In heavy nasal carriers of *Staph. aureus*, the hands are often infected, and there may be suppurative skin lesions on the hands and arms. Measures little short of a surgical aseptic technique may have to be taken to prevent infection of the ingredients, and stringent regulations should be laid down about the adequate heating and subsequent refrigeration of the food.

The consumption of any animal food in the raw condition is attended by a certain amount of risk. This is particularly true of milk and cream. There is general agreement that, for the prevention of food poisoning and other milk-borne diseases, these articles should be submitted to pasteurization or other adequate form of heat treatment.

BOTULISM

Introductory

Botulism (from Latin *botulus*, a sausage), sometimes known as allantiasis, or ichthyosismus, is a disease that was first described in 1820 by the German poet and medical writer Justinus Kerner (Jordan 1917). The causative bacterium was isolated and described by van Ermengem in 1896 (1896, 1897). Following on a festive gathering of a music club at the village of Ellezelles in Belgium, several of the members were taken ill, and three of them died within a week. The disease was confined to those who had partaken of a certain piece of raw ham; the other ham and the remainder of the animal had been previously consumed without causing trouble. The contaminated ham, which was paler and softer than normal and smelt rancid, had during the process of pickling been placed at the bottom of the vessel and had come into contact with the brine. When fed to cats it caused mydriasis, partial paresis, secretory disturbances, aphonia, and other symptoms. From it van Ermengem cultivated a strictly anaerobic organism, which secreted a powerful toxin, giving rise to the same symptoms in cats as those caused by the original ham.

Since that date several outbreaks of botulism have been recorded, some in Germany, but more in America, where of late years the disease has attracted general attention. In this country only four small outbreaks have been described. (See p. 1614.)

Symptomatology and Pathology.

The incubation period is generally under 24 hours after consumption of the affected food, but may be prolonged to 72 hours. Prominent among the symptoms are vomiting, constipation, ocular pareses, thirst, pharyngeal paralysis, the secretion of thick, viscid saliva, and sometimes aphonia. General consciousness and sensibility remain intact till near the end, which is preceded by coma or delirium. The temperature is generally subnormal—96–98° F. Later in the illness it may rise owing to the onset of broncho-pneumonia (Dickson 1918). Death may occur within 24 hours from the time of onset, or may be delayed for a week. In cases that survive, complete recovery, particularly of the ocular movements, may not take place for 6 or 8 months. At autopsy the kidneys, liver, and meninges are congested. Histological examination of the organs may reveal the presence of thrombosis.

How the toxin acts is still under dispute. Kempner and Pollack (1897), Marinesco, and several of the earlier workers found changes in the nerve cells of the brain and spinal cord of animals dying from *botulinum* poisoning. The work of Dickson and Shevky (1923) and Cowdry and Nicholson (1924), on the other hand, suggests that the main effect of the toxin is exerted on the peripheral nerve endings, possibly those of the autonomic system. More recent observations by Bishop and Bronfenbrenner (1936) point likewise to the myoneural junction

as the main point of attack, the toxin behaving like curare in paralysing the nerve endings. Contrary to the findings in tetanus, there appears to be no fixation of the toxin by the brain tissue (Coleman 1924).

Bacteriology.

Botulism is an intoxication, not an infection. The causative organism, *Cl. botulinum*, multiplies in the food before it is consumed, and produces a powerful soluble toxin, which on ingestion is absorbed by the mucosa of the stomach and upper part of the intestine, and gives rise to the characteristic disease. The potency of this toxin is such that the fatal dose for an adult man, calculated on the basis of animal experiments, might be as small as 1/100 mgm. or even less (Bengtson 1924). The smallest lethal dose of the strongest tetanus toxin that has yet been prepared is about 25 times as great as this. Its ability to pass through the gastric mucosa unchanged distinguishes it from tetanus or diphtheria toxin, both of which are harmless when given by the mouth. This may be due partly to its resistance to acids ; in an alkaline medium, such as prevails throughout the greater part of the small intestine, it is unstable. Of the two main types of *Cl. botulinum* that have been encountered in human botulism, Type A is much commoner than Type B ; thus in 23 outbreaks occurring in the United States, 19 were due to Type A, and only 4 to Type B (Editorial 1926). Two fatal cases due to Type E have been described in the United States, one following the consumption of German-canned sprats, the other of smoked Nova Scotia salmon (Hazen 1938).

Though the disease is essentially an intoxication, evidence has been accumulating that under certain conditions the organism may multiply to a limited extent in the animal body. It is not necessary to examine this evidence in detail, but briefly it has been found (Edmondson *et al.* 1920*b*, Orr 1920, 1922, Coleman and Meyer 1922, Graham and Eriksen 1922, Coleman 1923, Hall and Davis 1923, Starin and Dack 1925) that if spores, which have been freed of their toxin by repeated washing or by heating to 80° C. for half an hour, are injected subcutaneously into guinea-pigs in large doses—500 million or so—or are given by the mouth in still larger doses—about 2,000 million—they may give rise to fatal disease ; from the spleen, liver, kidneys, or mesenteric glands the bacilli may be recovered in pure culture after death. The experiments on which these findings are based are not very convincing ; doubtless after such massive doses the spores are disseminated throughout the body. But it is doubtful whether germination of the spores occurs or whether the resultant bacilli multiply and form toxin. Since it is extremely difficult to free spores completely from all traces of toxin, especially by washing, and since very small quantities are required to kill a guinea-pig—1/10,000 mgm. or less—it is not surprising that some animals injected with so-called toxin-free spores succumb to the disease.

Even if it is possible experimentally to reproduce a true infection with *Cl. botulinum*, there is no evidence that under natural conditions the disease in man is other than a pure intoxication. In this view the majority of authors strongly concur (van Ermengem 1897, Römer 1900, Armstrong *et al.* 1919, Burke *et al.* 1921, Geiger *et al.* 1922).

A disease closely simulating botulism in man can be reproduced by the experimental inoculation or feeding of cats, monkeys, and certain other animals with toxic material (see Chapter 36).

Epidemiology.

Botulism is due to the consumption of food in which *Cl. botulinum* has been growing. It occurs therefore in sporadic outbreaks limited to those who have

partaken of the contaminated food. Since the disease is an intoxication, not an infection, secondary cases do not occur, but it is common for cats, dogs, and especially chickens that are fed on the remnants of the food to develop symptoms of poisoning.

Botulism is not a common disease. From 1899 to 1925, 146 outbreaks were reported in the U.S.A. and Canada, affecting 504 persons; of these 337 died—a case mortality of 67 per cent. (Editorial 1926). Bacteriological evidence of the nature of the disease was available in 53 of these outbreaks. These figures probably underestimate the incidence of the disease; at any rate it appears to have become commoner during the last few years when greater attention has been focussed upon it. During the 8 years 1918–25 in the U.S.A. there were about 13 outbreaks per annum.

In Germany 24 outbreaks of suspected botulism were reported between 1907 and 1923, but in only one of these was *Cl. botulinum* isolated. In the British Isles only 4 outbreaks have been recorded, the largest of which occurred in 1922 at Loch Maree in Scotland, when 8 persons were attacked after eating potted duck paste; all the patients died within a week. *Cl. botulinum* Type A was cultivated from the remnants of the paste (Leighton 1923). The remaining three were in London. Two (Templeton 1935, Lane and Jones-Davies 1935), of which one was shown to be due to Type A, followed the consumption of vegetarian nut brawn. The third (Aitken, Barling and Miles 1936), which was due to Type B, occurred after eating a home-made meat pie. In America the disease is commoner during the winter months—the season of preserved food consumption.

Morbidity and Mortality Rate.—In any outbreak the morbidity rate is high. As a rule all who partake of the contaminated food develop the disease. The mortality rate varies in different outbreaks. In Germany it is said to be not more than 25 per cent. (Mayer 1913), but in America it is 60–70 per cent. (Burke *et al.* 1921). The earlier the symptoms appear, the higher is the mortality rate; of those that develop symptoms in 24 hours 84 per cent. die; of those that develop symptoms in 72 hours 55 per cent. die; and of those that are alive after the 8th day 20 per cent. die (Burke *et al.* 1921).

Type of Food.—Nearly all the reported outbreaks of botulism have been caused by food that has been smoked, pickled, or canned, allowed to stand for a time, and eaten before cooking, or after inadequate cooking (Jordan 1917). No cases have yet followed the consumption of fresh food, cooked or uncooked. In several instances those who have consumed the affected food after cooking have escaped, whereas those who have consumed it prior to cooking have been attacked (Geiger *et al.* 1922). There is a difference between Europe and America in the type of food responsible for poisoning. In Europe most cases have been due to sausages, ham, preserved meats, game pâtés, potted goose or duck, and brawn; a few, especially in Russia, have been ascribed to salted fish, such as salmon and sturgeon (van Ermengem 1897, Nitta 1919). In America, on the other hand, the contaminated foods have generally been canned fruits and vegetables, such as olives (Armstrong *et al.* 1919, Edmondson *et al.* 1920*a*), string beans (Geiger *et al.* 1922, Geiger 1924, Stricker and Geiger 1924), corn (Geiger *et al.* 1922, Geiger 1924), spinach (Geiger 1920), and peas (Editorial 1926). A few have been due to cooked meat or fish (Editorial 1926), and some to cheese (Nevin 1921). Home-canned string beans alone accounted for 19 out of 55 outbreaks in America (Editorial 1926).

In the majority of instances, the preserved foods have been noticeably spoiled.

The cans are often blown and show numerous gas bubbles on opening; the solid part of the food has a mushy or disintegrated appearance, and smells like rancid butter or cheese (Burke 1919*c*). In the case of ham the flesh is often paler and softer than normal, and has a rancid odour (van Ermengem 1897, Savage and White 1925*b*). The food, however, may be well preserved.

Mode of Infection of the Food and Distribution of Cl. botulinum in Nature.

The most extensive investigations on the distribution of *Cl. botulinum* in nature have been conducted by Meyer and his colleagues in America. It is abundantly clear that the organism is a normal inhabitant of the surface layers of the soil, from which it may obtain access to vegetables, fruits, hay, silage, and other cultivated produce. It may be transported by insects, and it may be eaten by horses, cattle, and other animals. In America it is common in the soils of the Western States of the Cordilleran system. It is less frequent in the Atlantic States, and is relatively rare in the Middle States, the Great Plains, and the Mississippi valley.

TABLE 150.

DISTRIBUTION OF *Cl. botulinum* IN DIFFERENT TYPES OF SOILS IN ALL STATES OF AMERICA, WITH THE EXCEPTION OF CALIFORNIA AND VIRGINIA.

Modified from Meyer and Dubovsky(1922*b*).

	Specimens Examined.	Toxic Cultures.	Weak Toxin.	Type A.	Type B.	Types A and B.
Virgin soil	335	105	22	59	22	2
Cultivated soils	274	47	13	18	16	—
Garden soils	142	41	12	23	6	—
Soil and manure from animal corrals and yards	161	20	9	6	5	—
Pasture	51	19	5	3	11	—

Tables 150 and 151, taken from Meyer and Dubovsky (1922*b*), illustrate the frequency with which it is encountered in different types of soils, and in vegetables and fodder.

In a similar study made of the soil and vegetable produce in California, 624 samples were examined; 206 yielded toxic cultures (Meyer and Dubovsky 1922*a*). Taking together the figures for the U.S.A. including California, Meyer and Dubovsky found that 581 of

TABLE 151.

DISTRIBUTION OF *Cl. botulinum* IN DIFFERENT VEGETABLES AND FODDER IN ALL STATES OF AMERICA, WITH THE EXCEPTION OF CALIFORNIA AND VIRGINIA.

Modified from Meyer and Dubovsky (1922*b*).

	Total No. Examined.	Positive Cultures.	Percentage of Positive Cultures.
Mouldy hay	44	7	15·9
String beans, pods and stalks	44	14	31·8
Decayed vegetation	29	6	20·6
Ensilage	15	3	20·0
Corn husks, leaves and stalks	80	6	7·5
Beets, roots and tops	37	6	16·2
Tomato plant and roots	24	2	8·3

2,162 samples examined showed the presence of *Cl. botulinum*, *i.e.* 26·8 per cent. Three hundred and ninety-five strains were typed; 250 or 63·3 per cent. were Type A, 138 or 34·9 per cent. were Type B, and 7 or 1·8 per cent. were mixed A and B.

From Table 150 it will be noticed that *Cl. botulinum* was commoner in virgin soils and pasture than in cultivated soils and manure. This has been held to indicate that the organism is a natural inhabitant of the soil and not merely a contaminant derived from animal fæces. Indeed the older view that the organism is a natural inhabitant of the intestine of animals, and is spread by their fæces, must be abandoned.

Meyer and Dubovsky (1922*c*) likewise examined specimens of soil from Belgium, Denmark, the Netherlands, England, and Switzerland. *Cl. botulinum* Type B was demonstrated in a variable proportion of soils from the different countries, but in not a single instance was Type A found. From this country 64 specimens were examined, and 9 of them yielded toxic cultures; the counties furnishing the positive results were Durham, Derbyshire, Middlesex, Hereford and Sussex, thus indicating a widespread distribution of the organism. In Scotland, Leighton and Buxton (1928), in an examination of 100 samples of soil, encountered *Cl. botulinum* four times; two of the cultures belonged to Type A, one to Type B, while the remaining one contained both Types A and B. Haines (1942), who examined 106 samples of soil from different parts of England—mainly the south-east—isolated *Cl. botulinum* with certainty from 5 per cent. Of the 5 strains, 4 came from grassland; 4 were Type A and 1 Type B. Both types of *Cl. botulinum* have been demonstrated in the soils of Canada, China, and at least one of the Pacific Islands (Dubovsky and Meyer 1922*b*, Schoenholz and Meyer 1922).

Meyer and Dubovsky's results have not as yet received general confirmation; some of their conclusions may have to be modified (Geiger and Benson 1923, Bachmann and Haynes 1924), and more work must be carried out before the relationship of the two types to environmental conditions can be definitely determined.

Cl. botulinum is present occasionally in the fæces of animals such as pigs, cattle, and horses (Burke 1919*a*, Tanner and Dack 1922, Easton and Meyer 1924), which feed on soil produce, but there is no evidence that it is a natural intestinal inhabitant. It is rarely present in the fæces of healthy human beings; Easton and Meyer (1924) failed to find it in 88 specimens examined, even though it was known that the spores were being ingested on raw fruits and vegetables used as food. Kahn (1924) likewise obtained negative results when examining the anaerobic flora of the stools of 72 persons.

Factors influencing the Development of Cl. botulinum in Canned Foods.—It is difficult to understand why, with such a wide distribution of *Cl. botulinum* in the soil and on fruits and vegetables, botulism itself is so uncommon. The organism is unable to grow in the animal body and give rise to infection; fresh foods, cooked or uncooked, may therefore be eaten with impunity. For botulism to result, the organism must multiply and form its toxin in the food before consumption. But even so one would expect the incidence of the disease from canned foods to be far greater than it is. We know that the spores of *Cl. botulinum* may withstand boiling for from half an hour to 22 hours (Bigelow and Esty 1920, Weiss 1921, Esty and Meyer 1922, Tanner and Twohey 1926), and a temperature of 120° C. for as long as 20 minutes. In the Loch Maree outbreak the potted duck paste had been heated during the process of manufacture at three different stages, the temperature in one stage approximating to 115° C. for 2 hours. Heat alone is therefore not sufficient to explain why the majority of canned foods are innocuous. Other factors must be concerned.

The hydrogen-ion concentration of the food has an important bearing on the efficacy

of sterilization. Esty and Meyer (1922) found that at pH 7·0 the spores were destroyed in 330 minutes at 100° C.; at pH 5·05 in 45 minutes, and at pH 3·7 in 10 minutes. The higher the hydrogen-ion concentration, the shorter is the time required for destruction (Bigelow and Esty 1920, Weiss 1921). The presence of sodium chloride in the food lowers the thermal resistance, which decreases with increasing concentration of the salt (Weiss 1921). The smaller the number of spores in the food the shorter is the time necessary to kill them (Bigelow and Esty 1920). Spores from young cultures are said to be more resistant to heat than those from old (Weiss 1921, Esty and Meyer 1922, Tanner and Dack 1922). These are some of the factors influencing the sterilization of the food.

It has also been found that spores of *Cl. botulinum* may remain dormant for days, weeks or months before germinating (Dickson *et al.* 1925). The type of food influences the development of the bacilli; string beans, spinach, corn, peas, and salmon appear to be specially favourable for growth; acid fruits and vegetables are less so (Koser *et al.* 1921, Geiger and Benson 1923, Schoenholz *et al.* 1923, Bachmann 1924). Below pH 4·5 germination is inhibited in most fruits. Such products can safely be processed in boiling water, whereas above pH 4·5 the processing must be sufficient to destroy even the most resistant spores (Esty 1935). Dissolved tin, such as results from the use of unlacquered cans, is said to inhibit growth (Scott and Stewart 1944). The presence of other organisms, such as *Cl. sporogenes*, apparently interferes with development (Jordan and Dack 1924). Finally, not all strains of *Cl. botulinum* form toxin (Bengtson 1922*b*) though the majority appear to do so (Starin 1924).

It is probable that a large number of factors determine the survival, germination, multiplication, and toxin production of *Cl. botulinum* in canned food. Unless these factors are favourably combined the food remains innocuous. The most important of these appear to be (1) the presence of spores in suitable numbers, (2) insufficient heating, (3) anaerobic conditions, (4) too slow cooking, (5) the use of the food without final cooking. The records make it clear that the danger from home-canned food is far greater than from that which is commercially prepared.

Given very favourable conditions, *Cl. botulinum* may produce detectable toxin within 12 hours, but usually it takes 2–14 days, depending on the temperature of storage of the food and other factors. According to Tanner and Oglesby (1936), and Tanner, Beamer and Rickher (1940), little or no growth occurs below 10° C., but food stored above this temperature may become dangerous fairly rapidly.

Diagnosis.

The symptoms of botulism in man are generally so characteristic as to render the disease capable of being diagnosed on clinical grounds. To confirm this diagnosis the following procedure should be adopted.

A. Demonstration of Botulinum Toxin in the Suspected Food.—For rapid diagnosis the food is suspended in saline, and injected intraperitoneally into three mice. A few hours before the injection, one mouse is given subcutaneously a small dose of antitoxin Type A and another Type B. Alternatively the food suspension may be added to the antitoxin, and the mixture injected subcutaneously. If the control mouse and one of the protected mice die, it may be provisionally concluded that *botulinum* toxin was present in the food, corresponding to the type of antitoxin which was given to the surviving mouse.

Meanwhile the food should be seeded into 2 per cent. glucose broth, Hitchens' medium (0·2 per cent. dextrose infusion broth containing 0·1 per cent. agar), beef heart peptic digest liver broth, peptonized bullock's heart broth (de Lavergne and Abel 1925), or cooked meat medium, and incubated anaerobically for 10 days at

35° C. The culture is then filtered, and the filtrate tested by intraperitoneal injection of mice or subcutaneous injection of guinea-pigs; antiserum Type A and B should be administered to two of the animals in order to determine the type of toxin present.

B. Isolation of the Bacillus from the Food.—Pure cultures may be obtained by heating broth cultures to 80° C. for half an hour to destroy contaminating non-sporing bacilli, and seeding into deep agar cultures, which are incubated anaerobically, preferably in the presence of added CO_2. The characteristic colonies should be picked off, grown in liquid medium, and tested for toxin production (Burke 1919*b*). Single colonies may likewise be obtained by plating the broth culture, after heating, on 5 per cent. horse blood extract agar plates incubated anaerobically (Wheeler and Humphreys 1924).

C. Demonstration of Botulinum Toxin in the Blood and Tissues.—Toxin may occasionally be demonstrated in the circulating blood during life. Schneider and Fisk (1939) report one successful instance in a case that later proved fatal, though they were unsuccessful in 3 non-fatal cases. The same authors succeeded in demonstrating the presence of toxin in the liver of 3 fatal cases at autopsy.

D. Isolation of Cl. botulinum from the Patient's Fœces or Vomit.—It is worth while attempting to culture the organism from the patient's fæces or vomit; the same method should be employed as for culturing it from food. Wheeler and Humphreys (1924) obtained pure cultures from the fæces of a woman on the 6th day of disease. Positive results have also been reported on fæces taken from the 6th to the 12th day of the disease (Dubovsky and Meyer 1922*a*).

E. Isolation of Cl. botulinum from the Intestinal Contents and Viscera at Autopsy.—*Cl. botulinum* does not usually invade the body, but it may occasionally be isolated from the liver (Dubovsky and Meyer 1922*a*) or spleen (Stricker and Geiger 1924).

F. Other Methods of Examination.—In patients that recover, antitoxin corresponding to the type of the organism concerned may sometimes be demonstrated in the serum. Thus Stricker and Geiger (1924) found antitoxin present in the serum of two patients on the 14th day after the consumption of the affected food; 0·25 ml. of the serum protected mice against 15,000 M.L.D. of Type A toxin, but not against Type B toxin.

Prophylaxis.

Sufficient information is now available about the conditions governing the destruction of *Cl. botulinum* in commercially canned foods to enable processing to be carried out satisfactorily. No outbreaks of botulism have, in fact, been caused since 1925 by foods preserved in American canning factories (Tanner, Beamer and Rickher 1940). Home-canning, on the other hand, is generally acknowledged to be fraught with danger, since the temperature attained in heating is as a rule too low to destroy the spores. Even fractional sterilization, which is possible in the home, is unreliable, because the spores may not develop in the meantime (Burke 1919*c*). In most outbreaks the food has been abnormal in appearance and odour. Bulging of the tin, the presence of gas bubbles on opening, a disintegrated appearance of the food, and a rancid smell, should be sufficient to condemn the food. On no account should such food be eaten, even after cooking (Tanner and Twohey 1926). But in some toxin-containing cans no evidence of spoiling is detectable (Schoenholz *et al.* 1923).

Prevention of botulism due to the consumption of made-up meat foods is particularly difficult. Van Ermengem (1897) lays emphasis on the necessity in pickling hams of using strong brine, which is unfavourable to the development of the organism.

In handling suspected food care should be taken not to allow it to come in contact with cuts; Geiger (1924) states that the toxin may be absorbed from broken skin areas, mucous surfaces, and fresh wounds.

Treatment.

The toxins produced by Types A and B of *Cl. botulinum* are antigenically distinct. Antitoxic sera can be prepared by the injection of rabbits (Nevin 1921), goats (Kempner 1897, Forssman 1905) and horses (Leuchs 1910) with each type of toxin. The antitoxin neutralizes the toxin according to the law of multiple proportions (Kempner 1897). Its therapeutic properties are limited. Injected before or simultaneously with the toxin it is protective for guinea-pigs and mice. Kempner (1897) found that 300,000 neutralizing doses, given 24 hours after the injection of the toxin, saved the life of a guinea-pig. Similar results were obtained by Kempner and Pollack (1897). Toxin-antitoxin mixtures given by the mouth are said to be dissociated in the stomach (Leuchs 1910). No international standard has yet been laid down for the antitoxin, but the intravenous inoculation of mice with toxin-antitoxin mixtures appears to be a promising method of titration (Glotowa and Dankerowitz 1935).

In the treatment of human cases antiserum has not yet been proved to be effective. Some favourable results have, however, been reported (McCasky 1919, Geiger 1920). Large doses, 50 ml. or more, of polyvalent serum, or of monotypical serum if the type of the intoxicating organism is known, should be given intravenously every day till the patient recovers, or all hope is abandoned. A prophylactic dose of 10 ml. should be given intramuscularly to all who have partaken of the poisonous food, and who have not yet developed symptoms of the disease. The antitoxin unit in the U.S.A. is that amount which prevents death within 4 days of guinea-pigs weighing 250 gm. injected with 1,000 M.L.D. of toxin.

Burke, Elder and Pischel (1921) state that liquid soap neutralizes the toxin, and that olive oil prevents its absorption from the gut. They advise therefore the use of high enemas of soap and olive oil. Iodine and potassium permanganate are both able to destroy the toxin *in vitro*, and might reasonably be given by the mouth. Alcohol precipitates the toxin (Armstrong *et al.* 1919); and it is possible that frequent small doses of brandy might prove beneficial. Mutch (1937) draws attention to the value of medicinal kaolin in the treatment of food poisoning on account of its ability to adsorb toxin. It is doubtful, however, whether any of these methods is likely to be of value in practice, since sufficient toxin will already have been absorbed by the time treatment is begun.

Botulism in Animals

Of recent years considerable attention has been devoted to a study of diseases of domestic animals characterized as a rule by fairly sudden onset, paralytic symptoms and often death. In many instances it has been possible to demonstrate a relationship between the type of fodder used and the occurrence of the outbreak. Organisms of the *botulinum* type have frequently been isolated from the animals and from the fodder, and the toxin produced in culture has been shown to be capable of reproduc-

ing the symptoms of the disease when given by the mouth or inoculated subcutaneously. The complete chain of evidence necessary to incriminate these organisms in the causation of the disease has often been lacking, but there seems to be little question that in many outbreaks the diagnosis of botulism has been essentially correct.

Great confusion exists about the exact identity of the various organisms isolated. Because some of them differ in their toxin production from the classical *Cl. botulinum* A or B types, the names *Cl. parabotulinum*, *Cl. parabotulinum bovis*, or *Cl. parabotulinum equi* have been suggested, and the disease caused by them has been termed parabotulism. This is not the place to discuss bacteriological nomenclature, but we are in entire agreement with Weinberg and Ginsbourg (1927) that for the moment these organisms should be regarded as varieties of *Cl. botulinum* and referred to as *Cl. botulinum* Type C, D, or E. Their relationship to each other and to the two classical types is very uncertain, and apart from slight differences in the nature of the toxin produced, there seems to be nothing that would justify their elevation to specific rank. Moreover, since some of the recorded outbreaks have been traced to toxin produced by the classical A or B types, and others in the same species of animal to toxin of the C, D, or E types, there seems to be no justification in referring to the former group as instances of botulism and to the latter as instances of parabotulism.

A complete study of the immunological behaviour of the toxins produced by these various organisms has not yet been made. The limited information available seems to show that the toxin of Types A and B is lethal, when given *per os*, to monkeys, ducks, horses, and laboratory animals, but probably not to goats. Type C_α is toxic by the mouth to ducks, slightly so to laboratory animals, but non-toxic to monkeys and goats. Type C_β is toxic by the mouth to monkeys, goats, ducks, and slightly so to laboratory animals. Type D is toxic by the mouth to goats, horses, and slightly so to laboratory animals, but not to monkeys. Type E is toxic by the mouth to horses. Both Types D and E seem to be very closely related to Type C (see Theiler *et al.* 1926–1927, Theiler 1928, Robinson 1929*a*, *b*, Gunnison and Meyer 1930, Gunnison and Coleman 1932).

Forage Poisoning in Horses.—Forage poisoning is a very common disease in America, affecting several thousand horses annually; numerous cases have been recorded in Europe and in Egypt. Buckley and Shippen in 1917 observed that experimentally induced botulism in horses closely simulated naturally occurring forage poisoning. From toxic oat-hay and ensilage they isolated a bacillus resembling *Cl. botulinum*; experimental intoxication with this organism could be prevented by the administration of anti-botulinum serum. Graham and Brueckner (1919) isolated an organism like *Cl. botulinum* from a corn ensilage that had been responsible for an outbreak of forage poisoning. Horses fed with 2 ml. of a broth culture of this organism manifested clinical symptoms of forage poisoning—difficulty in mastication, ptyalism, muscular weakness, pharyngeal paresis, recumbency, dyspnœa, protruding and pendulous tongue, mucous discharge from the nostrils, marasmus and enuresis—and died in a week. At necropsy lesions similar to some of the gross lesions occurring in forage poisoning were observed. Horses given a prophylactic dose of *botulinum* antiserum on the previous day recovered. On the other hand, Gaiger (1924) in this country has found that horses are comparatively insusceptible to *Cl. botulinum*, whether the culture is given by mouth or inoculated into the tissues. In view of the widespread distribution of *Cl. botulinum*, it is

obviously insufficient to demonstrate its presence in the fodder, or in the intestinal canal of the animal ; it must be shown that the contents of the intestine are definitely toxic.

In South Africa Theiler (1928) made some interesting observations on forage poisoning in mules. Circumstances surrounding the death of two mules in the same manger suggested that the food had become contaminated. Examination revealed the presence of a semi-decomposed rat in the hay, and bacteriological investigation left no doubt that the carcass of the animal contained *botulinum* toxin which had contaminated the hay. The part played by rats in the spread of botulism in horses and cattle is dealt with by Theiler and Robinson (1927) and Robinson (1929*a*). The organism isolated in South Africa is referred to as the E type, but it is closely related to the C_β type of America and Australia.

Forage poisoning is a wide term covering a number of different conditions, and it is not to be thought that it is uniformly due to poisoning with the toxin of *Cl. botulinum*. According to Walker (1929), the grass disease of this country is almost certainly different from botulism, while according to Gordon (1934) a disease in some respects resembling grass sickness may result from intoxication with products of *Cl. welchii* (see Chapter 36).

Botulism in Cattle and Sheep.—Forage poisoning in cattle is commonest between the ages of 6 months and 2 years ; the mortality is 2–10 per cent. (Graham and Schwarze 1921*b*). From a corn silage that was shown to have been responsible for an outbreak in cattle on two different occasions, Graham and Schwarze (1921*b*) isolated *Cl. botulinum* Type B. Whether the organism had any causal relationship to the poisoning was not definitely ascertained.

Dubovsky and Meyer (1922*a*) isolated *Cl. botulinum* from the liver and mesenteric glands of two cows suffering from ictero-hæmoglobinuria. In Australia Seddon (1922) has investigated an epizootic disease of cattle known by the names of Midland cattle disease, impaction paralysis, or dry bible ; this disease appears to be identical with the *lamziekte* of South Africa. From animals dying of the disease he isolated an organism, which he called *B. parabotulinus* ; this organism is now referred to as the C_β type of *Cl. botulinum*. Experimentally its toxin gave rise to bulbar paralysis in cattle.

Our knowledge of botulism in cattle has been extended by Theiler and his colleagues (1926–27) working on lamziekte in South Africa. This disease, which is characterized by paresis and paralysis, principally of the locomotor system, but sometimes of the muscles of mastication and deglutition, occurs in areas where the phosphorus content of the soil, and hence of the pasturage, is deficient. According to Theiler, the affected animals have a craving for phosphorus. This they endeavour to satisfy by eating the debris of carcasses, especially bones, which they find on the veld. Some of these carcasses, or the remains of muscle around the bones, happen to be infected with *Cl. botulinum*, and if sufficient toxin has been produced by the multiplication of this organism, the animals are liable to develop the characteristic symptoms of lamziekte (see also Seddon 1927). The disease can be prevented by feeding bone-meal to the animals ; this satisfies their craving for phosphorus, and so prevents their indulging in the so-called practice of " osteophagia." The infecting organism appears to be mainly of the D type, but, as already noted, it is closely related to the C_β type found in Australia by Seddon. According to Scheuber (1929), it is found in the soil near decomposing carcasses, and often in the intestinal contents of both sick and healthy animals.

Bennetts and Hall (1938) find that botulism of cattle and sheep in Western Australia results largely from the eating of carrion containing the toxin of Type C. Experimentally, they were able to show that as little as 3–5 gm. of toxic rabbit carrion might prove fatal to sheep. The length of time that carrion remains toxic varies with the rainfall and other factors, but sometimes it may be as long as six months. *Vaccination* with two doses of alum-precipitated *botulinum* toxoid at an interval of not less than two months conferred on both cattle and sheep a high degree of immunity.

Limberneck in Chickens and Duck Disease.—In 1918 Dickson made the following statement: " In the series of outbreaks (of botulism) which I have investigated it has been the rule to find that varying numbers of chickens have died from limberneck." This disease is characterized by weakness, muscular incoordination, a drooping neck, anorexia, prostration, and coma. Death frequently follows. Geiger, Dickson and Meyer, writing in 1922, were able to collect records in the U.S.A. of 103 outbreaks since 1910 with 3,500 deaths. Evidence that limberneck is an avian type of botulism is rapidly accumulating.

The toxin of *Cl. botulinum* has been demonstrated in the crop and intestinal contents of fowls dying of the disease (Graham and Schwarze 1921*a*, Graham and Boughton 1923*a*, Geiger *et al.* 1922). Experimentally it has been found that the feeding of toxic material gives rise to symptoms of limberneck (Dickson 1918). The disease has frequently followed the consumption of home-canned string beans (Meyer and Dubovsky 1922*b*). Most of the outbreaks investigated have been due to Type A, but there is evidence that Type B may be toxic to chickens (Geiger *et al.* 1922).

In 1920 Wilkins and Dutcher concluded from their experiments that limberneck was a disease resulting from paralysis following the ingestion of fly larvæ (*Lucilia cæsar*). Two years later Bengtson (1922*a*, 1923) isolated from the larvæ an organism similar in many respects to *Cl. botulinum*, which in large doses reproduced the symptoms of limberneck. This organism she called *Cl. botulinum* Type C (1923). These findings were confirmed by Graham and Boughton (1923*a*, *b*). It would appear therefore that chickens may develop limberneck from ingestion of fly larvæ containing *Cl. botulinum*; the evidence, however, is as yet insufficient to establish this conclusively.

A disease of **ducks** has been reported in different parts of the world (see Gunnison and Coleman 1932) which appears to be a form of botulism. Several large outbreaks have occurred in Canada and the United States. *Cl. botulinum* Type C has been isolated from the liver, and the corresponding toxin has been demonstrated in the bodies of ducks dying from this disease. Intoxication appears to be due to the consumption of food material in which the organisms have been multiplying, or to the ingestion of infected fly larvæ. Quortrup and Holt (1941) and Quortrup and Sudheimer (1943) have made some interesting observations on the ætiology of the disease. They find that *Cl. botulinum* Type C can grow well and produce toxin in decaying vegetation in the duck marshes. Growth is favoured by the presence of *Ps. pyocyanea*, which uses up oxygen and reduces acidity, and is hindered by certain other organisms which produce acid fermentation or destroy the toxin (see Sherman, Stark and Stark 1927). Whether an epidemic occurs among the ducks or not in any given year depends upon the balance in Nature between the favouring and the inhibiting factors.

Sporadic cases of botulism have been reported in a number of *other animals and birds* (see Robinson 1929*b*). Outbreaks of poisoning in cats, dogs, and goats have followed the consumption of home-canned string beans (Geiger *et al.* 1922). Experimentally, Graham and Eriksen (1922) found dogs to be resistant to feeding with *botulinum* toxin, but to be susceptible to Type A, not to Type B, toxin on subcutaneous injection.

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CHAPTER 73

PLAGUE, PASTEURELLOSIS, AND PSEUDOTUBERCULOSIS

PLAGUE

Introductory

Plague has been one of the greatest scourges of the human race. From time to time it has swept over the world in relentless waves, exacting a toll of life probably unequalled by that of any other epidemic disease. In the 5th and 6th chapters of the 1st Book of Samuel there is an unmistakable account of bubonic plague. Before the Christian era 41 epidemics are on record; during the 1,500 years following the birth of Christ there are records of 109 epidemics, including the great plague of Justinian's reign, and the Black Death of the fourteenth century. Between 1500 and 1720 there were 45 pandemics of plague (Editorial 1925*a*). During the eighteenth and the nineteenth centuries it was comparatively quiescent, being confined almost entirely to endemic foci in various parts of Asia; but at the close of the last century it sprang once more into activity. Starting in Hong-Kong in 1894, it invaded India, Japan, Asiatic Turkey, and European Russia in 1896; the following year it reached Madagascar and Mauritius. In 1899 Arabia, Persia, the Straits Settlements, Austria, Portugal, British South Africa, Egypt, the French Ivory Coast, Portuguese Africa, the Argentine, Brazil, Paraguay, and the Hawaiian Islands were affected. There was a small outbreak at Glasgow in 1900, and in the same year the disease appeared at Sydney in Australia and at San Francisco, California. Undoubtedly the brunt of the disease was borne by India, where in the 20 years 1898–1918 more than 10 million deaths were recorded (White 1918–19).

At the present time plague is spread widely but unevenly throughout the world. One of the most disturbing features is the progressive infection of rodents that is occurring in America, South Africa, and apparently certain other countries; these animals form a reservoir for the conservation of the plague bacillus.

The causative organism was isolated independently and almost simultaneously by Kitasato (1894) and by Yersin (1894), at Hong-Kong in 1894. Numerous workers, particularly Ogata (1897), Simond (1898), and Gauthier and Raybaud (1902, 1903) were responsible for showing that the disease is primarily one of rodents, and that it is spread to man by the agency of infected fleas. This conception was criticized by several workers (Nuttall 1898, Galli-Valerio 1900, 1903), but was definitively proved to be correct by the English Plague Commission (Report 1906).

Epidemiology.

There are certain parts of the world from which plague never dies out; these constitute permanent endemic foci, and serve as starting-points for epidemics.

The maintenance of infection in these areas depends on the rodent population. In the South-Western Himalayas it is the rats that are infected, in the Kirghiz Steppes it is the spermophiles, in Transbaikalia the tarbagans, in California the ground squirrels, and in South Africa the gerbilles. Periodically epidemics of plague break out amongst these rodents, exterminating a large proportion of them. For some time infection lies dormant, manifesting itself only by isolated deaths. Then later, as the animals breed and a fresh susceptible population arises, another wave occurs, leading again to decimation of the herd (Elton 1925).

Man is infected from these animals either directly or, more often, by the intermediation of fleas. Year by year, single cases of plague, or groups, occur, small in themselves but sufficient to show that the disease is still smouldering. So long as these foci remain secluded they are of little importance; but once communication is set up with the surrounding country, there is a danger that plague may take on epidemic spread.

An epidemic starting in this way runs a characteristic course. In the pre-epidemic phase there are a few cases separated by considerable intervals of time. This is succeeded by the epidemic phase in which a rapid succession of cases occurs constituting the major portion of the outbreak. Finally there is a decline resembling the commencement, but not so long drawn out. Infection does not occur by direct contact with the sick; it spreads by contiguity from place to place, so that an increasing number of small foci are established. Often the infection seems to be localized to buildings, particularly grain stores, warehouses, or shops; in these, many cases of plague are contracted by people who have no immediate relation to each other. Plague has a predilection for dirty, insanitary dwellings, and more particularly for their ground floors; it attacks the lowest class of the population living under the worst conditions.

Infection may also occur *per saltum*. A focus of plague suddenly appears several miles—sometimes hundreds of miles—from the primary focus, and serves in its turn as the centre from which infection may spread to the surrounding localities. The contiguous mode of spread is dependent on the gradual dissemination of plague amongst the rats; *per saltum* infection results either from the transportation of rats or rat fleas by railways, ships, or other means of communication, or by human beings infested with rat fleas. It is probably due to importation of plague from cities that villages are infected. Greenwood (see Report 1911, p. 47) obtained evidence that villages situated near railways were more liable to plague than villages not so situated.

Though bubonic plague has occurred in nearly every part of the globe, it is confined chiefly to the warmer latitudes (Robertson 1923). Extreme heat and dryness of the atmosphere are inimical to its spread; thus in India it occurs during the colder months of the year, when the mean temperature is between 50° F. and 85° F., and the air has a high relative humidity, or, as it is sometimes expressed, a low saturation deficiency. According to White (1918–19), humidity is the most important factor in the spread of plague in India; the years of maximum mortality have been the years with a high mean relative humidity. This was particularly true of the Punjab and Northern India (White 1918–19).

The case mortality of bubonic plague is about 60–90 per cent., of pneumonic plague 100 per cent.

In Bombay the English Plague Commission (Report 1907, p. 724) determined

that prior to the annual outbreak of human plague there was an epidemic amongst the rats. First the rats belonging to the species *Rattus norvegicus* (*Mus decumanus*) were affected. After an interval of about 10 days an epizootic appeared in the rats of the species *Rattus rattus* (*Mus rattus*); and after a further interval of 10 days the human epidemic broke out. From this they inferred that *R. rattus* was infected from *R. norvegicus*, and subsequently conveyed the disease to man. It was further observed that plague persisted in *R. norvegicus* during the off-season—in Bombay from June to December—and flared up at the onset of the colder weather (Fig. 286, p. 1630).

The transference of plague from rat to rat and from rat to man occurs almost exclusively by fleas. Chief amongst these are *Xenopsylla cheopis* and *Ceratophyllus fasciatus*—the rat fleas. It has been shown that in the absence of their specific hosts, both of these types of flea will bite human beings. Plague is essentially a septicæmic disease; towards the end of an attack the bacilli are present in the blood in enormous numbers, and are readily imbibed by the fleas that infest the rat. When the animal dies, the fleas leave the corpse and wait for a suitable opportunity to attach themselves to a fresh host. Meanwhile the bacilli multiply in the proventriculus, often to such an extent as to block it completely, and prevent access of food to the stomach. A flea in this condition is hungry, and when it succeeds in finding a new host, attacks it with vigour. The act of sucking, however, only distends the already contaminated œsophagus, and on the cessation of the pumping act some of the blood is forced back into the wound (Bacot and Martin, see Report 1914). Sometimes a temporary passage is cleared through the mass of obstructing bacilli; this fails, however, to restore the lost valvular function to the proventriculus; it merely leaves a passage through which the blood can flow out of the stomach as freely as it enters. Hence after a full meal, blood extends from the posterior portion of the stomach to the anterior chamber of the pharyngeal pump. Such a flea is probably more dangerous than one whose proventriculus is completely blocked, since the contents of the stomach can be regurgitated into the wound with greater freedom (Report 1915*b*).

The length of time that a flea can remain *infected* depends on several factors, chief of which are temperature and humidity. In India fleas were found to harbour plague bacilli up to 47 days (Report 1915*a*), in Madagascar for several weeks (Girard 1936), and in the United States for as long as 130 days (Eskey and Haas 1940). Survival of the bacilli in the flea is favoured by a low temperature—about 50° F.—and a nearly saturated atmosphere; adverse factors are a temperature over 80° F., or an even lower temperature with a dry atmosphere (Liston 1924). But the time that fleas can remain *infective* is much shorter; most of them die in a day or two, and the limit of infectivity is probably about 7–14 days.

It has been suggested that the bacilli in the flea's stomach may be attacked by the phagocytes in the rat's blood which has been ingested; when the temperature rises the phagocytes become more active. This may explain why a flea clears itself more quickly at a high than at a low temperature, and also why, if it is fed on healthy blood after being infected, it clears itself more quickly than when it is starved. The clearance is said to be still more rapid when the flea is fed on the blood of immunized animals (Report 1908, p. 260). It is possible that in this manner the increasing proportion of immune rats towards the close of an epidemic exercises an inhibitory effect on the spread of the disease.

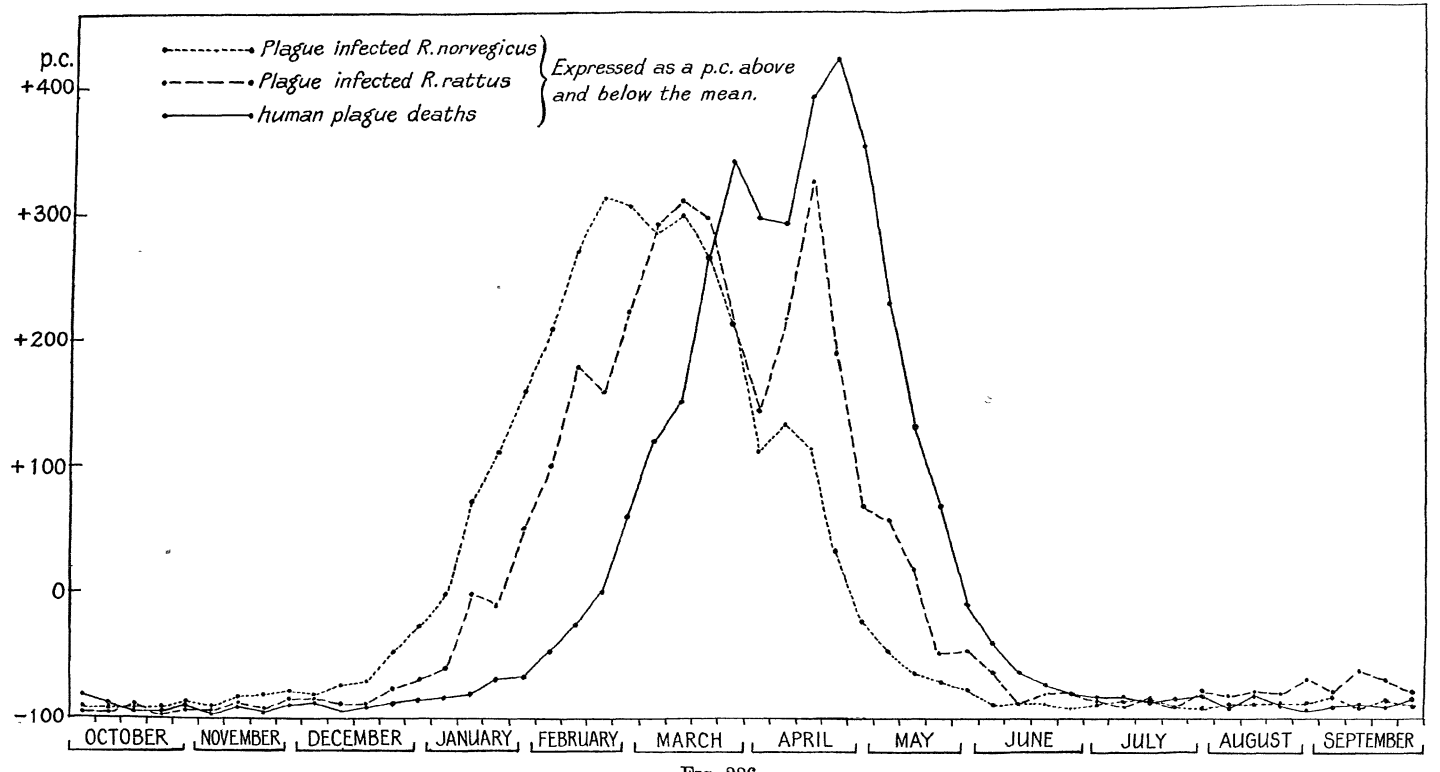


FIG. 286.

Chart illustrating the sequence of epidemic plague in rats and man. The figures are expressed as percentages above and below the mean. (After the English Plague Commission in India.)

Plague in Rats.

In India the two species mentioned above are chiefly responsible for the spread of plague. *Rattus norvegicus* is the large grey rat; it lives in sewers, stables, and garbage. *Rattus rattus* is the black rat; it is smaller and less fierce than the grey rat, and, living in houses or their immediate neighbourhood, it comes into closer contact with the human population. The natural mode of infection of rats appears to be almost entirely by fleas. It is possible that animals may acquire the disease by devouring their dead companions; but considering that it is not easy to infect rats by feeding on plague material, and that the post-mortem appearances in rats dying naturally of plague are different from those of rats experimentally infected by feeding, it is doubtful whether this is more than a rare occurrence.

In Bombay, of the dead rats examined during 1905–06, 22·2 per cent. of *R. norvegicus*, and 16·7 per cent. of *R. rattus* were infected with plague. Of the live rats the figures were 0·85 per cent. and 0·37 per cent. respectively. Thus plague was commoner in *R. norvegicus* than in *R. rattus* (Report 1907, p. 724). The susceptibility of the two species to experimental inoculation with plague was almost identical. In Sydney during $2\frac{3}{4}$ years ending December 31, 1904, 125,872 rats and mice were caught and examined; plague was identified in 0·37 per cent. (Thompson 1906).

During the height of the epizootic the lesions are those of acute plague, but during and subsequent to the decline, a number of healthy rats are encountered with atypical lesions. One of the commonest of these is a large abscess in the spleen or liver, containing plague bacilli. The rats, though infected, have evidently been able to withstand the attack. Swellengrebel and Hoesen (1915) in Java, and Bordas, Dubief and Tanon (1922) in France found a number of rats with atypical lesions containing plague bacilli of lowered virulence; on passage through one or two guinea-pigs they became fully virulent again. Williams and Kemmerer (1923) in New Orleans confirmed the existence of *latent plague* in rats. It has also been found among other rodents in Russia and the United States. Whether these animals act as chronic carriers of the disease and are responsible for keeping alive the infection from one epizootic to the next is still an open question. Most workers are disposed to attribute more weight in this respect to the survival of plague bacilli in the flea rather than in its host (Girard 1936, Eskey and Haas 1940).

Experimental Transmission of Plague in Rats.—It has been abundantly demonstrated by different workers that plague does not spread from rat to rat by contact in the absence of fleas. On the other hand infection spreads readily in the presence of fleas, even though the animals have no immediate contact with each other. Two experiments of the English Plague Commission will illustrate this.

EXPERIMENT 1.—(Report 1910.) Bombay rats were inoculated with plague and mixed with normal rats in godowns. In one set of godowns fleas were excluded, but in the other set they were permitted to multiply. In the flea-free godowns none of the normal rats, which were in continuous contact with the infected rats, developed plague, whereas in the others infection spread rapidly (Table 152).

The rats dying of plague in the flea godowns nearly all showed cervical buboes, indicating that the site of infection had been on the head or neck; this is the situation on which the fleas chiefly congregate.

EXPERIMENT 2.—(Report 1906, p. 421). This was a repetition of an experiment performed by Gauthier and Raybaud (1902, 1903). Two wire-mesh cages were employed,

TABLE 152

EFFECT OF FLEAS ON THE MORTALITY OF RATS IN EXPERIMENTAL EPIDEMICS.

	Flea-free Godowns.	Flea Godowns.
Inoculated rats put in	84	70
„ „ died of plague	73 or 86·9%	63 or 90%
Normal rats put in	125	125
„ „ died of plague	0	57 or 45·6%
Plague rats eaten	3	1
Fleas present	None	About 24 per rat

each standing in a shallow tray filled with dry earth or sand. The cages were enclosed in a single glass box, the roof of which was covered with fine muslin impervious to fleas (see Fig. 287). Rats were introduced through the lids of the cages, and the lids themselves were covered with fine muslin. Into Cage X was placed a rat, inoculated with plague, together with 10 to 20 fleas. As soon as the rat was dead, a healthy rat was placed in Cage Y. The carcass of the first rat was allowed to remain in X for 8 to 12 hours after the animal's death. During that time fleas transferred themselves to the new rat through the wire mesh of the cages. Immediate contact between the two rats was absolutely prevented. This experiment was repeated several times, and in 69 per cent. of instances in which healthy, presumably non-immune, rats were used, the rat in Cage Y contracted plague.

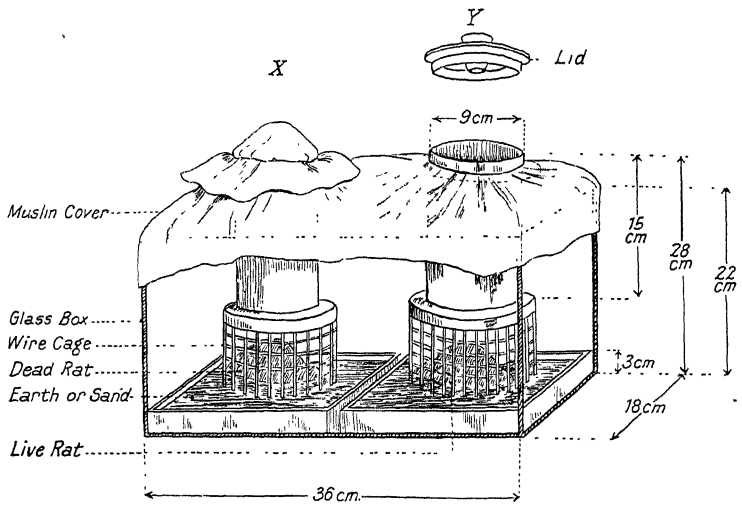


FIG. 287.

The wire-mesh employed was 3 mm.; in the figure it is shown much larger, for the sake of clearness.

Experiments were also carried out on guinea-pigs (Report 1906, p. 421). A series of 6 godowns were specially constructed by the Commission. The walls were of brick and mortar, 9 inches thick; the floors were of concrete on top of a high plinth. Each godown measured internally 7 ft. × 6 ft. Leading into the interior were double doors, lined with wire netting, between which was an inspection chamber. The essential difference in the structure of the godowns was in the roofs. Nos. 1 and 2 were roofed with country tiles, placed in four layers on the top of wooden laths. On the inside of this roof there

was a wire netting on a wooden framework in Godown 2, and two layers of wire netting, 10 inches apart, in No. 1. So that while rats could build their nests in the tiles of the roof, they were completely shut off from the interior of the godowns. Mangalore tiles were used for roofing Godowns 3 and 4; these do not afford so good a shelter for rats as country tiles; a single layer of wire netting separated the tiles from the interior. Godowns 5 and 6 were roofed with a single layer of corrugated iron fastened down with cement to the tops of the walls, so that no rats could nest at all. Godowns Nos. 1 and 3 had a small roof light; the rest were in darkness (Fig. 288).

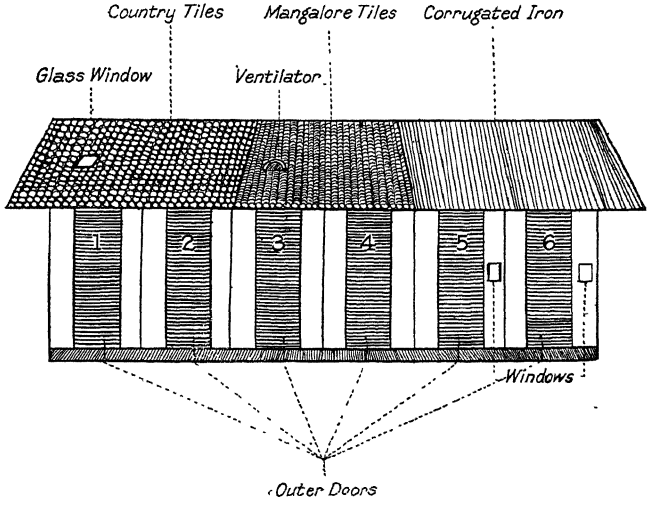


FIG. 288.

The doors opened into the left-hand side of each godown, not in the middle as indicated in the figure.

By allowing 3 guinea-pigs to run about in each godown for 6 days, and making flea counts every day, it was ascertained that the number of fleas varied in each instance with the accessibility of the roof to rats. Thus in No. 1, 54 fleas; in No. 2, 228; in No. 3, 40; and in No. 4, 70 fleas were caught in 6 days. In Nos. 5 and 6 fleas were few or absent. Incidentally this experiment brings out the importance of darkness in favouring infestation by fleas.

In these godowns a series of experimental epidemics was initiated amongst guinea-pigs, generally by introducing a number of animals experimentally infected with plague and adding normal animals to them. For the detailed results, reference must be made to the Commission's Report (1906); the conclusions that were drawn from them were as follows:

(1) Close contact of plague-infected with healthy animals, if fleas were excluded, did not lead to the production of an epizootic. Since the godowns were never cleaned out, close contact involved contact with fæces, urine, and discharges of infected animals, and the eating of contaminated food.

(2) Close contact of the young with the infected animals failed to infect the former; even the suckling of young animals by infected mothers proved ineffectual.

(3) If fleas were present, then an epizootic broke out, the rate of progress being in direct proportion to the number of fleas present.

(4) Infection can take place without any contact with contaminated soil. Thus, in one experiment, guinea-pigs placed in wire cages 2 inches above the ground developed plague. But when the cages were suspended 2 feet above the ground, none of them became ill, indicating that aerial infection does not occur.

In another experiment 2 monkeys were placed in Godown No. 2, in which 3 guinea-pigs had recently died of plague. Each monkey was in a separate cage of the same pattern, the only difference being that one was surrounded by a layer of tanglefoot (an adhesive substance) 6 inches wide, while the other one was unprotected. (A rat flea cannot jump farther than about 5 inches.) The roof of each cage was impervious to fleas, and the bottom was of teak wood, which prevented contact of the animal with the ground. After two nights in the godown the monkeys were removed and segregated. Two fleas were caught on the unprotected monkey, and five fleas were found stuck on the tanglefoot. The unprotected monkey soon became ill, developing a bubo in the right axilla, which contained plague bacilli. The protected monkey remained well.

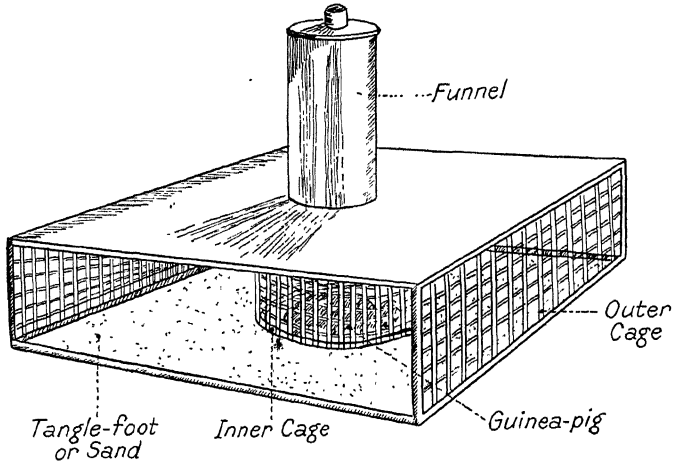


FIG. 289.

The outer large mesh wire netting, which has been omitted from one side of the outer cage, was to prevent the tanglefoot from being destroyed, and to prevent rats from coming into close contact with the guinea-pig. The tanglefoot prevented the access of fleas to the inner cage; the sand did not.

Equally suggestive were the experiments in the plague houses of Bombay. Guinea-pigs were allowed to run about in these houses to act as traps for fleas; the usual number of fleas—mostly rat fleas—caught in this way was about 20 per room. In 29 per cent. of instances guinea-pigs left in these rooms for from 18 to 40 hours contracted plague. The situation of the bubo was usually in the cervical region—explicable by the habit that fleas have of collecting round the under surface of the neck.

In other experiments guinea-pigs were placed in cages, of the pattern shown in Fig. 289. One of the cages was surrounded by a 6-inch boundary of tanglefoot; the other by a layer of sand. When left in plague-infected houses, 24 per cent. of the animals in the unprotected cages developed plague whereas not one of the animals in the protected cages did so.

Mode of Spread of Plague in Bombay.—The conclusions drawn from these experiments receive support from epidemiological observations in the field. Plague in man predominates amongst the lowest classes, particularly those in dirty and

insanitary dwellings, where rats and fleas are numerous. The higher classes, such as the Parsees and the Europeans, suffer very slightly. The presence of an initial bubo renders it highly probable that infection occurs by the skin, and the predominant selection of the inguinal region for the development of the bubo suggests that the bacilli gain entrance through the skin of the leg In confirmation of this it has been noted by several observers that in about 5 per cent. of cases the first sign of plague is a small vesicle, surrounded by an area of redness (Simond 1898). This is found generally on the leg, and is undoubtedly due to the bite of a flea. Living plague bacilli are said to be uniformly present in these vesicles.

Though rats in Bombay breed throughout the year, both *R. rattus* and *R. norvegicus* breed most freely from June to October. The largest proportion of young rats in relation to the total population is reached in November and December.

Rat fleas are most prevalent during the months of February, March, April, and May. The infestation of *R. norvegicus* is more than double that of *R. rattus*. Thus the number of fleas per rat during these months is about 5 for *R. rattus* and 12 for *R. norvegicus*. In this connection it will be remembered that plague is commoner in *R. norvegicus* than in *R. rattus*. The epidemic season for rats and man lasts from January or February till April or May, the maximum prevalence being either in March or April. The sequence of events would appear to be as follows. During the hot season the rats breed freely, and a large susceptible population is produced. As the colder weather arrives, the fleas increase in numbers, and plague breaks out in *R. norvegicus*. This is rapidly followed by an epizootic in *R. rattus*, and this in its turn by the human epidemic. At the onset of the hot weather in May or June the flea population decreases rapidly, and plague in rats and man comes to an end for the year. The subsidence of the epidemic is accounted for partly by the increasing proportion of immune rats, and partly by the decrease in the number of fleas. With regard to the former it was found by the English Commission that Bombay rats captured towards the end of the epizootic were more resistant to experimental inoculation with *Past. pestis* than those at the beginning. Whether the rats that had survived the epizootic were more resistant *ab initio*, or had acquired immunity during the progress of the epizootic, it was impossible to say.

During the off-season sporadic plague occurs in rats, particularly *R. norvegicus*, and in man; but the conditions are obviously unsuitable for its spread on any but a small scale.

We have dealt at some length with the conditions prevailing in Bombay, because they have been investigated so thoroughly. In other parts of India much the same sequence of events has been observed, with, however, variations dependent on climatic and other factors. It is curious that 75 per cent. of the plague cases in India are distributed over the Punjab, the Bombay Presidency, and the United Provinces, when it is remembered that these areas contain less than a third of the total human population (Low 1920). This anomaly is explicable largely by the favourable climatic conditions existing in these areas for multiplication of fleas. Where these conditions are unsuitable, plague either does not occur or runs a diminutive course. Madras, for example, though bordering on the highly infected Bombay Presidency, is relatively free from plague. The probable reason for this difference is that the predominating rat flea in the Madras Presidency is

X. astia, which as Liston (1924) and Hirst (1926, 1927) have shown, is considerably less effective in carrying plague from rat to rat than *X. cheopis*. A supplementary, but probably less important, reason is that during about 7 months of the year the climatic conditions in certain parts of Madras are unfavourable both to flea reproduction and to the transmission of infection by rat fleas. It is noteworthy that in Madras and Colombo plague seems to be confined to areas in which *X. cheopis* is prevalent. While *X. astia* is indigenous to Southern India, *X. cheopis* has only been recently introduced—mainly by the grain and cotton trade. It is still spreading, or attempting to spread, in these new areas, and the incidence and severity of plague epidemics appear to be associated directly with the number of *cheopis* present (King and Pandit 1931).

The conclusions advanced by the English Commission (Report 1908, p. 266) on the seasonal prevalence of plague in Bombay and the Punjab were as follows: The *rise* of the rat epizootic, and therefore of the human epidemic, depends on (1) a suitable mean temperature, somewhat below 85° F. and in general over 50° F.; (2) a sufficient number of susceptible rats; (3) a sufficient number of rat fleas.

The *fall* of the rat epizootic and of the human epidemic depends on (1) a high mean temperature, 85° F. or over; (2) a diminution in the total number of rats and an increase in the proportion of immune to susceptible animals; (3) a diminution in the number of rat fleas.

In Egypt, Petrie and Todd (1924) were able to determine the influence of season on the spread of the disease. In the Southern Provinces the maximum incidence was in February or March; in Northern Egypt, at Alexandria and Port Said, it was in July. The optimum temperature for epidemic spread was 68°–77° F., and the optimum vapour pressure deficiency 1–10 millibars. These, too, were the optimum conditions for the development of fleas. There was a lag period of 4 to 6 weeks between the onset of favourable atmospheric conditions and epidemic prevalence of the disease in man; this was apparently taken up by the development and multiplication of *X. cheopis*. The two rodents chiefly concerned in the spread of plague were *Rattus rattus* and the Cairo spiny mouse (*Acomys cahirinus*). *X. cheopis* constituted from 90–100 per cent. of the fleas on these animals trapped in houses.

In the mountainous districts of Java, according to Otten (1932) fleas reach their maximum in October and November, and human plague reaches its maximum in December. The flea index follows the rise and fall of the saturation deficiency—just the opposite to what occurs in India.

It will be gathered that the conditions determining the epidemic spread of plague are very stringent. The links in the rat-flea-man chain must be delicately adjusted; a slight fault in one link is sufficient to impair the efficiency of the whole chain, or even to break it altogether. This is doubtless the reason why in countries where the conditions are not so favourable as in India, plague often has great difficulty in spreading (Robertson 1923). Several times during the present century it has been introduced into England, chiefly at the ports, yet it has uniformly failed to take hold. In East Suffolk, it is true, it is endemic in the rats (Bulstrode 1910–11, Eastwood and Griffith 1914, Macalister and Brooks 1914), having been imported probably in grain; but apart from attacking one or two persons it has shown no potentialities for spreading to the human population (see Greenwood 1935).

Plague in other Rodents.

GROUND SQUIRRELS AND OTHER AMERICAN RODENTS.—Plague first appeared at San Francisco in 1900. By 1904 it had spread from the rats to the ground squirrels—*Citellus beecheyi*—and had begun to pass eastward from the coast (McCoy 1910). In 1934 it was found that plague had crossed not only the central valleys of California, but also the Sierra Nevada mountains into the Great Basin section of Oregon. A new species of ground squirrel, *Citellus oregonus*, was shown to be infected. As a result of special field surveys it was learnt that by 1940 no fewer than ten States had been invaded. Three groups of rodents appear to constitute the main primary reservoirs of infection in the west: (1) ground squirrels in the coastal regions and northern part of the inter-mountain plateau; (2) wood rats in the southern deserts; and (3) white-tailed prairie dogs in the plateau regions of Arizona and New Mexico. A more limited reservoir is furnished by chipmunks, mantled ground squirrels, and marmots. Numerous species of fleas, lice, and ticks are concerned in the transfer of infection among these rodents (Eskey and Haas 1940).

Cases of human plague have been traced to contact with ground squirrels, but they have been few. During the ten years, 1930–39, only 8 cases were reported (Hampton 1940). If, however, plague should cross the Rocky Mountains and infect the urban rats of the middle west, a more serious situation might arise. (For a description of the progress in combating what is sometimes referred to as "sylvatic plague" in the western part of the United States the reader is referred to a series of articles by Meyer [1936, 1937, 1938, 1939].)

GERBILLES AND MULTIMAMMATE MICE.—Plague was introduced into South Africa in 1900–02 by rats in forage vessels coming from South American ports. Since then isolated outbreaks have occurred in different parts of the country without any explanation being apparent of the mode of infection. In 1921, however, a case of plague on a farm was traced to infected rodents. It was discovered that the gerbilles or Nachtmuis (*Gerbillus tateronia*) and the multimammate mice (*Rattus coucha*) in the locality were infected with plague (Mitchell 1921). Both of these animals come into fairly close contact with man. Plague spread rapidly in the rodents during 1923–24, and in that year there were 167 outbreaks of human plague in the Union, with 372 cases and 235 deaths. Large epizootics have occurred, killing off 80–90 per cent. of the veld rodents and 30–40 per cent. of the associated small carnivora—mongoose and suricat. The rodents are not completely exterminated by the disease; the survivors breed freely, so that in time another large susceptible population appears, which is likewise decimated by plague (Kauntze 1926). It is estimated that plague is distributed over about 50,000 square miles (Editorial 1925*b*).

SPERMOPHILES AND FIELD MICE.—Endemic plague in the Steppes of South-west Russia has been traced to spermophiles—*Spermophilus musicus* and *Sp. rufescens*—and to field mice. In winter when the spermophiles are hibernating, cases of human plague appear to be due to infection from wandering field mice, which take refuge in the thatched roofs of the houses (Zabolotny 1923). A number of cases in winter, however, appear to be of the pneumonic type (Radcliffe 1879–80). In certain provinces, such as Saratow, Astrakhan, and the Don province, marmots are also infected (Tschurilina 1930).

TARBAGANS.—Numerous outbreaks of plague have occurred in Transbaikalia, Mongolia, and Manchuria. The origin of these has been traced to infected tar-

bagans (Zabolotny 1923, Petrie 1924, Wu Lien Teh 1924). The tarbagan (*Arctomys bobac*) is a small rodent living in deep burrows. From September to April it hibernates, and the breeding season is in July and August. During April, May, June and September its skin is largely sought after by Russian and Chinese hunters for the manufacture of imitation sable. The tarbagan harbours fleas—*Ceratophyllus silantievi* Wagner—which will bite man when starved (Wu Lien Teh 1913). In its burrows there are certain passages used for the deposition of excrement, and the corpses of animals that have died during hibernation, as well as skin, bones, and other refuse. These passages are in absolute darkness, and are cool, moist, and of equable temperature; it is probable that they afford suitable conditions for conservation of the plague bacillus during the winter (Jettmar 1923).

During the summer, sporadic cases of bubonic plague occur amongst the tarbagan hunters. In the winter pneumonic plague occurs in epidemic form.

Pneumonic Plague.

The epidemiology of pneumonic plague is different from that of the bubonic form, one of the chief differences being that the former is highly contagious, whereas the latter is not.

Provided the conditions are favourable, primary pneumonic plague may spread with great rapidity. In 1910–11 the outbreak in Manchuria and North China levied a toll of 60,000 lives; in 1917–18 there were 16,000 deaths in South Mongolia and China, and in 1920–21 there were 9,000 deaths in Transbaikalia (Wu Lien Teh 1922). The epidemic is independent of rodents; it spreads directly by droplet infection from one person to another. The closer the contact, the more likely is infection to spread; this accounts for the prevalence of pneumonic plague in the winter, when overcrowding in insanitary dwellings is so common. This form of the disease appears to be uniformly fatal (Jettmar 1923).

In some countries, such as Manchuria, Transbaikalia, and the Kirghiz Steppes, bubonic plague occurs during the warm weather, and pneumonic plague during the winter. Under these conditions it seems probable that the outbreaks of pneumonic plague arise from cases of bubonic plague complicated by secondary pneumonia.

Bacteriology and Diagnosis of Plague in Man and Animals

Diagnosis of Natural Rat Plague.

During an epizootic the diagnosis of plague in rats can be made almost as well macroscopically as by microscopical examination. The signs and their frequency in *R. rattus* and *R. norvegicus* combined were as follows (Report 1907):

	Per cent.
(1) Subcutaneous congestion, particularly in submaxillary region	30·5
(2) Subcutaneous hæmorrhages, particularly in submaxillary region or flank	40·5
(3) Cervical œdema	10·0
(4) Single bubo	73·05
(*a*) Cervical bubo — 75 per cent. of cases of single bubo	
(*b*) Axillary bubo — 15·1 „ „ „ „ „	
(*c*) Groin bubo — 6·1 „ „ „ „ „	
(*d*) Sublumbar bubo — 3·8 „ „ „ „ „	

	Per cent.
(5) Multiple buboes	11·67
(6) Granular liver	55·5
(7) Pleural effusion	64·5
(8) Hæmorrhages in lungs and pleuræ	24·0
(9) Hæmorrhages in kidneys and suprarenals	8·5
(10) Granular spleen	4·5

Without doubt the presence of a bubo is the most important single indication of plague. In the early stage the gland is enlarged, congested, and shows hæmorrhagic points on section. When fully developed, it contains an area of grey necrosis confined to the medulla, or occupying the whole gland. The bubo is hard, and can be moved about under the skin with ease. Microscopically, plague bacilli are found in about 99 per cent. of buboes; in over half the buboes involution forms are present. The bacilli are best demonstrated by staining with carbol-thionin; with this dye they are faintly coloured except at the poles, which stain deeply.

The liver frequently shows a patchy distribution of alternate red and yellow areas—best described as mottling. In many instances there are small, grey or whitish areas of necrosis, giving the organ a stippled appearance as if it had been dusted with grey pepper; as a rule they do not project above the surface. The spleen is sometimes enlarged, and is firm and moulded over the stomach; sometimes it contains granules or actual nodules, which may be discrete or confluent. Occasionally a wedge-shaped portion of the spleen is converted into a cheese-like mass. Pleural effusion is a very characteristic feature; it is clear, abundant, and straw-coloured, less often blood-stained. The five most important signs of plague are: (1) bubo; (2) subcutaneous and general congestion; (3) granular liver; (4) congested spleen; (5) pleural effusion. Of these 1, 3, and 5 may persist in rats that have undergone putrefaction, and are then of great diagnostic value.

Microscopically, bacilli are most abundant in the bubo, then in the spleen, and least numerous in the heart's blood. Particular attention should be paid to the polar-stained barrel forms in fresh material, and to the characteristic rings and discs in putrefying material (Kister 1930). Cultures should be made from these three situations, single colonies picked off, and carefully studied.

Inoculation of guinea-pigs or mice should be made subcutaneously with a suspension of the bubo or spleen. If the animal has undergone putrefaction, and other organisms are likely to interfere with the inoculation test, pieces of the tissue should be rubbed on to the shaven abdomen of a guinea-pig. *Past. pestis* is able to penetrate the unbroken skin, whereas very few other organisms can do so. The Commission found that this test failed in only 2 per cent. of fresh and 10 per cent. of putrid rats.

In putrid animals the *thermoprecipitin test* is sometimes of value (Kraus 1897*b*, Piras 1913, Warner 1914, Philip and Hirst 1917). One part of the finely divided organ is mixed with 5 to 10 parts of distilled water, boiled for 5 minutes, filtered repeatedly through paper or asbestos wool under pressure till clear, and run on to the top of a specific serum in a small tube. In a positive reaction a precipitate appears in 5 minutes at 37° C., increasing to a maximum after 2 hours.

There are several other diseases that may be confused with plague.

Pasteurellosis in rats does not appear to be very common, but when it does occur it is most confusing (Smillie 1920, Wayson 1925; see also Mitchell 1930).

The bacilli are smaller, more regular, and differ from plague chiefly in their sugar reactions and in their formation of indole.

Past. pseudotuberculosis (rodentium) gives rise to an infection in rats often accompanied by the formation of nodules in the spleen and liver. As a rule these nodules are larger than those of plague, and stand out more from the surface. One means of differentiation is the injection of the organisms into white rats; these animals are said to be susceptible to plague but relatively resistant to pseudotuberculosis. *Past. pseudotuberculosis*, if examined in broth cultures after about 16 hours' incubation at 22° C., is generally found to be motile, whereas *Past. pestis* is uniformly non-motile. (For further differences between these organisms see Chapter 32.)

According to Levinthal (1931), buboes in both rats and guinea-pigs may be caused by *Bact. coli*. Differentiation is readily accomplished by growth on MacConkey agar. Agglutination of the organisms with an anti-plague horse serum is not always reliable, because the serum may contain agglutinins to *Bact. coli*.

Infection of rats with *Trypanosoma lewisii* may readily be confused with plague (Macalister and Brooks 1914). Diagnosis is easy on microscopical examination, since trypanosomes are found in large numbers in the blood.

The lesions of plague in other rodents are similar to those in rats, and need not be described separately. In ground squirrels there is a disease called Tularæmia, which closely resembles plague; cultivation is necessary to distinguish between them.

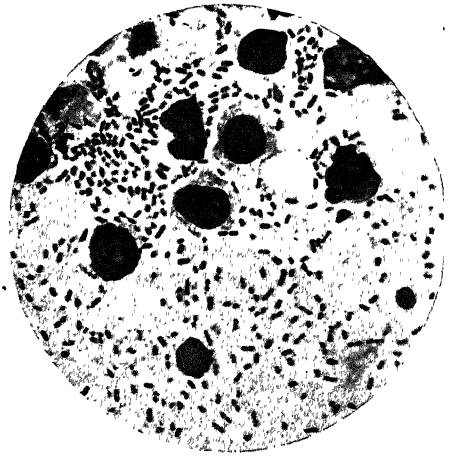


FIG. 290.—*Pasteurella pestis*.
Film from inguinal bubo of infected guinea-pig, showing the bipolar-stained ovoid bacilli (× 1000).

The diagnosis of epidemic plague in rats is comparatively simple, but great difficulties are met with in the sporadic form. The lesions are often atypical (Eastwood and Griffith 1914), buboes may be absent, and sometimes nothing characteristic may be noticed. Williams and Kemmerer (1923), in an examination of rats at Galveston, Texas, found 9 plague-infected rats, none of which showed any macroscopic signs of plague. The infection was discovered by gross or combined inoculations of the tissues of all rats in each day's catch. They advise that the rats should be divided into batches of ten, and the combined spleens or glands of each batch should be emulsified and injected into a guinea-pig. Even this method is not infallible, because at the decline of an epizootic or during an inter-epizootic period the virulence of the bacilli may be so low that the guinea-pig either does not die or dies with atypical lesions; in this case a fresh inoculation should be made from the guinea-pig, and repeated in series till the virulence of the organism is sufficiently exalted to give rise to typical plague. In the search for plague infection among ground squirrels and other similar rodents it has been found more satis-

factory to collect the fleas from the animals and inoculate them in pooled batches into guinea-pigs (Eskey and Haas 1940).

When a hitherto uninfected locality is invaded by plague, the rats may succumb rapidly to a septicæmic form of the disease, in which buboes are insignificant or absent (Philip and Hirst 1917). Such rats may appear almost normal to the naked eye, though the bacilli are seen microscopically in large numbers in the blood and viscera.

Towards the end of an epizootic, lesions of chronic or resolving plague may be found. The chief of these are: (1) chronic buboes; (2) necrotic areas in the spleen; (3) chronic abscesses in spleen, and more rarely in liver; (4) perisplenitis; (5) scars in spleen; (6) bisected or trisected spleen; (7) adhesions of spleen to surrounding structures. In these lesions bacilli may or may not be found.

Diagnosis of Plague in Man.

During Life.—A small vesicle is sometimes present on the leg in the early stages of the disease corresponding to the flea-bite. Microscopical examination of the fluid will show the presence of plague bacilli. The bubo, which is generally in the inguinal region, should be aspirated, and the fluid examined microscopically, by culture, and by animal inoculation (Fig. 290).

Plague bacilli are frequently present in the patient's blood, where they may be demonstrated microscopically or, preferably, by culture. In the early stages of the disease 34 to 60 per cent. of blood cultures have been reported positive by different observers; but Bonebakker (1936), making use of Kirschner's (1934) observation that pure bile constitutes an excellent medium for the growth of plague bacilli in blood, was able to demonstrate their presence in 85 per cent. The degree of bacteræmia is said to fluctuate considerably during the course of the disease (Report 1908, p. 221).

The German Plague Commission (Report 1899) found that the patient's serum often agglutinated the plague bacillus, though usually to a low titre 1/2 to 1/40. A positive test is of value, a negative one meaningless. In mild or chronic cases, when the diagnosis is in doubt, and the bubo is small, hard, and difficult to aspirate, the bubo should be excised and injected into a guinea-pig (Uriarte 1925). In pneumonic plague large numbers of bacilli are found in the sputum.

The fæces of plague patients do not contain *Past. pestis*; the urine often does (Ogata 1897)—in 19 to 30 per cent. of patients (Report 1908, p. 221).

After Death.—Microscopical, cultural, and animal inoculation tests should be relied on for diagnosis, the best material being the bubo or the spleen. If the cadaver is comparatively fresh, the tissue suspension should be injected subcutaneously or intraperitoneally into a guinea-pig; the latter method is more reliable if the organisms are few. In putrid cadavers the organ should be rubbed on to the shaven abdomen of a guinea-pig or on to its nasal mucosa (Zlatogoroff 1904, Girard 1934); the thermoprecipitin test may likewise be used. In frozen cadavers *Past. pestis* can be cultivated from buboes up to 102 days (Zlatogoroff 1904).

Immunity.

There is evidence that after plague has been epidemic among rats for a time it decreases in incidence and ultimately dies out. This process is associated with an increase in the immunity of the rats. It has been shown that rats from a plague-infected area are more resistant to experimental inoculation than those

from non-infected areas; and the young of resistant animals are said to enjoy a high degree of natural immunity (Liston 1924).

Sokhey and Chitre (1937), for example, in India collected 80 rats from each of a number of towns that had suffered in varying degrees from plague during the period 1899–1929, inoculated each of them with a standard dose of virulent plague bacilli, and observed the resulting mortality. They found that, in general, the proportion of animals dying was inversely proportional to the total human plague incidence in the town from which they were derived. Thus, in Bombay city, where plague had been prevalent, only 7·9 per cent. of the rats died; in Madras city, on the other hand, where there had been practically no plague, 91·1 per cent. died.

How far resistance is due to an increased genetic immunity in the strict sense, resulting from the operation of natural selection, and how far it is due to the acquirement of active immunity by the young animals as the result of latent infection, occurring perhaps at a time when antibody derived from the placental circulation or the colostrum is still present in the animal's tissues, it is difficult to say, but readers who are interested in this fascinating problem will do well to consult the review of the subject by Hill (1934). On the whole, the evidence points more to the importance of genetic immunity. For example, in Bombay city, over 2,000 rats were caught each night and examined at the Haffkine Institute. During two years not a single plague-infected rat was found (Sokhey and Chitre 1937). Again, in the region of Paris, where plague was introduced by infected rats in 1917, the resulting epizootic gradually declined after about 1920, so that by 1936 not a single rat out of 3,525 examined was found to be infected (Joltrain 1936). In view of these findings it is difficult to ascribe the increasing immunity of the rats to the persistence of a benign epizootic. It seems more probable that a strain of rats is evolved having a greater degree of natural resistance to plague infection than that to which the bulk of the original susceptible animals belonged.

Prophylaxis, Vaccination, and Serotherapy

Prophylaxis.—The aim of prophylaxis is to weaken or break the rat-flea-man chain by striking at one of its links. The rat constitutes the first link. Complete destruction of all rats and mice is, however, quite impracticable. The animals breed at such a rate as to render futile all attempts at extermination. According to the English Plague Commission the progeny of a single pair might number 858 in 16 months (Report 1911). Thompson in Sydney found that, in spite of an intensive campaign against rats and mice, the number caught each week was nearly uniform; there was no evidence that the slaughter policy was making any real impression on the general horde. Again, complete destruction of rats on ships is impracticable; if performed thoroughly it would cause more dislocation of the traffic than would be tolerated. It has been suggested by Rodier (1926) that a more efficacious method would be to kill all female rats caught, and liberate the males; the resulting polyandry, he considers, would lead to practical extinction of the species.

Rats are comparatively harmless so long as they do not come into intimate contact with human beings. The most hopeful policy is to render all houses, granaries, shops, and such places unsuitable as rat habitations. That this can be done successfully has been demonstrated in Java, where between 1914 and 1939 over 1,500,000 houses were remodelled (van Loghem 1939).

The second link in the chain—the flea—must be dealt with by strict personal

hygiene and domestic cleanliness. In the absence of dirt and litter the fleas have nowhere to breed. The possibility that other insects—bugs, flies, ticks—may share in spreading the disease has been carefully considered (Nuttall 1897, 1898, Report 1915*c*). There seems little doubt that lice and ticks play a part in the transmission of sylvatic plague (Hampton 1940), but their rôle in the spread of plague among rats would appear to be negligible.

Man forms the third link. Four measures are usually advocated: (1) Notification of cases or, in countries where this is difficult, registration of deaths. The diagnosis can be confirmed post mortem by splenic puncture. (2) Isolation of cases, which, except in pneumonic plague, is of little value, since man plays but a small part in the spread of the disease. (3) Evacuation, which is sound in theory, since it removes the susceptible population from the infected localities, but is very difficult in practice—at any rate on a large scale. (4) Vaccination, the chief value of which is, or should be, to protect an exposed population while effective preventive measures, such as the remodelling of houses to exclude rats, are carried out.

Against pneumonic plague we are as powerless as against influenza or other rapidly spreading respiratory disease; the most important measures are the isolation of the patients and the prevention of overcrowding, especially at night.

Vaccines.—Animals were successfully vaccinated against plague by several of the early workers (Yersin *et al.* 1895, Report 1899, Report 1910, p. 536). Different species appear to vary in their response to a given vaccine. According to Schütze (1939*a*) a high content of envelope antigen is required in the vaccine if rats and guinea-pigs are to be protected, but in the mouse this antigen is of less importance. These observations may explain some of the discrepancies between the protagonists of dead and of living vaccines. Sokhey (Report 1936, 1937), for example, using mice has apparently obtained very good results in the laboratory by the use of a killed vaccine made from a broth culture grown at 27° C., which is known to contain very little envelope antigen; whereas Otten (1936, 1938) using guinea-pigs and rats, and Pirie and Grasset (1938) using rats, found a dead vaccine to be greatly inferior in its protective power to a living vaccine made from a strain of modified virulence.

In the prophylaxis of human plague a killed vaccine has been extensively used in India, but of late years the successful use of living vaccines in Java and Madagascar has seriously challenged the older method.

Dead Vaccine.—Haffkine's vaccine is prepared from a 4-weeks' culture of *Past. pestis* at 27° C. in goat digest broth, killed by heat for 15 minutes at 55° C., and preserved with 0·5 per cent. phenol. According to Schütze (1934), it is desirable to neutralize the alkali in the culture before heating in order not to destroy the small amount of envelope antigen in the bacilli. The vaccine is standardized by a mouse protection test (see Taylor 1933, Sokhey and Maurice 1935, Sokhey 1939). Four ml. is the usual dose for an adult, given at a single inoculation. The protective value of this vaccine is difficult to assess. Most of the trials have been made in India, where exact collection of statistics is notoriously difficult. The selection of the groups for vaccination has in some instances not been above criticism. To give an adequate summary of the results obtained would require more space than can here be spared; the reader who wishes to examine the evidence is referred to the Indian Plague Commission's Report (1901). A summary, however, of the more important groups of figures is reproduced (Table 153).

TABLE 153

HAFFKINE'S VACCINE. INCIDENCE AND MORTALITY OF PLAGUE IN INOCULATED AND UNINOCULATED PERSONS IN 14 OUTBREAKS. (Modified from Taylor 1933.)

Average Population at Risk.		Inoculated.		Uninoculated.	
Inoculated.	Uninoculated.	Attacks.	Deaths.	Attacks.	Deaths.
123,134	168,638	803	385	4,014	3,194
Attack rate per 10,000 . .		65		238	
Death rate per 10,000 . . .		31		189	
Case mortality		48 per cent.		80 per cent.	

These figures suggest that inoculation confers roughly a fourfold protection against attack, and a sixfold protection against death. The effect of the vaccine probably lasts for some months.

Living Vaccines.—Otten, in Java, has been the chief advocate of the living vaccine. Having convinced himself (see Otten 1933, 1936) that no dead vaccine could protect guinea-pigs or wild rats satisfactorily, he turned to a living vaccine made from a strain of modified virulence that had been originally cultivated from a plague rat at Tjiwidej. A single dose of this vaccine was found to protect 80 per cent. of rats and over 90 per cent. of guinea-pigs against inoculation with a dose of virulent plague bacilli that killed 99–100 per cent. of the control animals. For human beings the vaccine is prepared by washing off with saline a 2–3 day agar culture grown at 30° C. It can be kept for a month in the ice-chest without loss of potency. At first it was used in two sub-districts of Java, when alternate persons only were inoculated. The results of this controlled trial, in which observations were made over a period of 5 months after vaccination, are summarized in Table 154.

TABLE 154

OTTEN'S VACCINE. MORTALITY FROM PLAGUE IN INOCULATED AND UNINOCULATED PERSONS (Otten 1936).

Sub-district in Java.	Inoculated.			Uninoculated.		
	Number.	Deaths.	Mortality per 1,000.	Number.	Deaths.	Mortality per 1,000.
Bandjaran . . .	18,479	28	1·5	20,669	103	4·9
Batoedjajar . . .	18,956	10	0·5	24,088	110	4·6
Total	37,435	38	1·01	44,757	213	4·75

There were larger numbers of young children in the control than in the vaccinated group; since there is evidence that susceptibility to plague is less in the first six years or so of life than later, the odds were probably weighted somewhat against the vaccinated subjects. Even so the mortality per 1,000 was 4 to 5 times higher in the control group.

Later figures given by Otten (1941) show the fall in the number of deaths in the residency of Priangan. Vaccination of 94 per cent. of the population of two

TABLE 155

PLAGUE DEATHS IN THE RESIDENCY OF PRIANGAN.

1934.	1935.	1936.	1937.	1938.	1939.	1940.
20,522	10,107	4,296	2,543	1,368	1,002	247

million was carried out in 1935, and continued, including re-vaccination, during 1936–39 (Table 155).

The results are necessarily difficult to interpret, since it is impossible to know what fall in mortality would have occurred in the absence of vaccination. The fall, however, in this instance was remarkably rapid, and the figures taken in conjunction with those of Table 154 suggest strongly that vaccination played a not unimportant part.

In Madagascar a living vaccine has been employed, made from a strain E.V., which had become attenuated by monthly subculture for 4 years (Girard 1936). From 1921, when plague was introduced, the cases rose from 187 to reach 3,880 in 1933 (Girard and Robic 1938, 1942). In 1935 vaccination was begun, and was followed by a fairly rapid reduction in the incidence and mortality rate (Table 156).

TABLE 156

CASES AND DEATHS FROM PLAGUE IN MADAGASCAR (Robic 1938).

Year.	Number of Cases.	Number of Deaths.	Remarks.
1933–34	3,493	564	
1934–35	3,605	510	
1935–36	3,035	507	First vaccination campaign
1936–37	1,376	206	Second vaccination campaign
1937–38	596	85	Third vaccination campaign

As in Java, the fall after vaccination was started appears to have been greater than would have been expected in its absence.

Grasset (1941) in South Africa uses a mixture of the Tjiwidej and E.V. strains for the preparation of a living vaccine. The dose recommended is 1,000 million organisms. This gives rise to a moderate local swelling with some tenderness, but to little or no constitutional reaction.

It is impossible to assess the comparative value of dead and living vaccines in the prevention of plague. Nothing but a simultaneous trial of the vaccines on the same population, together with a proper control group of uninoculated subjects, is likely to supply us with the necessary information. There is little doubt, however, that provided due care is exercised to prevent contamination, the living avirulent vaccine can be employed without danger for the inoculation of human beings.

Serum Treatment.—A number of sera have been used in the treatment of human plague. Rowland (see Report 1911, p. 11) stated that serum prepared by immuniz-

ing horses with a solution of "nucleoproteins" from the plague bacillus had definite curative properties in rats. A report on the use of Yersin's, Haffkine's, Lustig's, Terni's, and Brazil's sera was published by the Government of India (Haffkine 1905); none of them affected the mortality of the disease. The English Plague Commission likewise obtained no evidence that serum treatment was beneficial (Report 1912, p. 326). More recently, both Girard (1936) and Pirie and Grasset (1938) have found that serum prepared by the inoculation of horses with living avirulent organisms is considerably more potent, as judged by animal tests, than that prepared from dead organisms. In human beings Girard (1941) states that 65 per cent. of patients suffering from bubonic plague recover under serum treatment. Of 70 patients treated with serum by Wagle, Sokhey, Dikshit and Ganapathy (1941), 71·5 per cent. recovered as against only 47·6 per cent. of 82 control patients receiving the routine iodine treatment. The serum was given in doses of 20 ml. intravenously and 20 ml. subcutaneously every day for 2–5 days. Alarming reactions may follow intravenous administration, and it might be better to give more frequent injections intramuscularly.

Chemotherapy.—Schütze (1939*b*), working with white rats and white mice, found that sulphapyridine had a protective effect similar to that of serum. In the series of patients just referred to, Wagle and his colleagues (1941) treated 53 patients with sulphapyridine and 32 patients with sulphathiazole in 0·5 gm. doses every 4 hours up to a maximum of 7 days; the recovery rates were 75·5 and 84·4 per cent. respectively. The results might have been better if a larger dosage had been tried. Neither serum nor sulphonamide seems to be of therapeutic value in pneumonic plague (Girard 1941, Wagle *et al.* 1941). According to Wayson and McMahon (1944), sulphadiazine is of undoubted therapeutic value in rabbits and guinea-pigs experimentally inoculated with plague. On the whole the evidence suggests that sulphathiazole and sulphadiazine are among the most active of the sulphonamide compounds.

PASTEURELLOSIS OR HÆMORRHAGIC SEPTICÆMIA OF ANIMALS

The observations of Bollinger and of Kitt in 1878 on an epidemic disease affecting wild hogs, deer, and later cattle in the neighbourhood of Munich constitute the first work of importance on a group of diseases attacking several species of animals, and known collectively as Hæmorrhagic Septicæmia. Kitt was successful in isolating the causative organism and in transmitting the disease to mice and pigeons. A similar organism was obtained by Pasteur in 1880 from fowl cholera, by Loeffler in 1882 from swine plague, by Poels (1886) from septic pleuropneumonia of calves, and by numerous workers during the next few years from diseases in other animals. In 1901 Lignières proposed to unite all these affections into one group under the name of Pasteurellosis, and to call the infecting organisms *Pasteurella*. He divided this group into 6 sub-groups according to the species of animal attacked:

(1) Avian type, attacking hens (fowl cholera), other birds, and rabbits.

(2) Porcine type, giving rise to swine plague (*Schweineseuche, Pasteurellose du porc*).

(3) Ovine type, affecting sheep (*pneumo-entérite, lombriz*), and infectious pneumonia of goats.

(4) Bovine type, attacking cattle (*Wild- und Rinderseuche, septicémie hémorragique des bovidés, pneumo-entérite du bœuf, barbone* of buffaloes, septic pleuropneumonia of calves, and *entiqué*).

The 5th and 6th sub-groups comprised equine influenza and canine distemper respectively, neither of which is actually due to *Pasteurella*.

This classification is incomplete and has little to recommend it; there is no place in it for pasteurellosis of the mouse, the rat, the reindeer and other animals that suffer naturally from the disease. Moreover it has now become clear that all true *Pasteurella* infections are manifestations of one infective process—Septicæmia hæmorrhagica. The causative organism is apparently the same throughout; and there is no means by which its animal origin can be ascertained with certainty.

The clinical manifestations of pasteurellosis vary according to the animal attacked; in some animals two or more forms of the disease have been recognized. Generally it runs an acute course with death in the space of a few hours to a few days. The mortality is high, 70–100 per cent. (Brimhall and Wilson 1900, Rice 1926). Post mortem, the main lesions are found in the viscera. Petechial hæmorrhages are common especially in the pleura, endocardium, and kidneys; and larger hæmorrhages may be scattered through the submucous connective tissue and lymphatic glands (Brimhall and Wilson 1900). In the pulmonary form the mucosa of the trachea and bronchi is congested; the lungs are hyperæmic and show diffuse or patchy consolidation; the pleura is covered with coagulated fibrin, and there is a fibrinous or purulent exudate in the pericardium (Poels 1886). The peribronchial and mediastinal lymph glands are swollen, and on section are moist and red. Congestion of the kidneys is noticeable, particularly in swine; microscopically there is epithelial necrosis of the tubules (Magnusson 1914). In the intestinal form the most noticeable feature is acute hæmorrhagic enteritis, with sometimes bloody mucus in the intestine (Rice 1926). The spleen and liver usually show little or no change (Voges 1896), but they are frequently covered with fibrino-purulent exudate, similar to that in the peritoneal cavity (Mørch and Krogh-Lund 1931). Microscopically the small, regular, oval, Gram-negative, bipolar-staining bacilli are found in enormous numbers in the blood and viscera. In the cow the udder may occasionally be affected (Lovell 1939).

The method by which the disease spreads is not altogether clear. Hueppe (1886) regarded it as a ground infection like anthrax, but the very low resistance of the organisms to inimical agencies would seem to exclude this. Magnusson (1914), who observed a large epidemic amongst reindeer in Sweden, suggested that it was spread by a sucking insect. This suggestion finds some support in the observations of Daubney, Hudson, and Roberts (1934), who brought evidence to show that the disease in cattle could be carried by the flea *Ctenocephalus felis* Bouche. Recently the belief has been gaining ground that it is primarily a respiratory infection. The organisms have been found on the mucosa of the mouth and in the nasopharynx of normal swine (Uhlenhuth and Haendel 1913). De Kruif (1921, 1922*a*, *b*, 1923), working with rabbits suffering from snuffles—a chronic form of the disease—observed the organism in the nose of a large proportion of normal animals. Webster (1924*a*, *b*) found that preceding an outbreak there was a rise in the normal carrier rate. The susceptibility of rabbits varied greatly, some being highly resistant, others succumbing with ease. Similar variation in the susceptibility of fowls to natural and experimental infection has likewise been

noted by Rice (1926). These observations suggest that natural infection occurs through the nose, and that it is dependent on the presence of carriers.

Pasteurella Infections in Man.—Though several authors have described diseases in man that they consider to be due to *Pasteurella* infection, few of the accounts carry conviction. Reviewing the literature, Regamey (1939) (see also Lévy-Bruhl 1938) has traced ten cases that were probably, and six cases which he thinks were certainly, due to this organism. Of the six genuine cases, three were characterized by pleurisy, two by meningitis, and one by a local lesion following the scratch of a cat. Subsequent cases have been recorded by Foerster (1938), Mulder (1938), Boisvert and Fousek (1941) and Ludlam (1944). Allott and his colleagues (1944) record six cases, three following cat bites and three following dog bites. Three of the cases were complicated by osteomyelitis. Localized disease is commonly followed by recovery, but generalized cases have usually proved fatal. Infection may occur without accompanying illness; in our own experience, for example, an animal house attendant who carried *Pasteurella septica* in his nose for several months remained quite well.

Protection, Vaccination, Serotherapy, and Chemotherapy.

It was when working with chicken cholera that Pasteur observed a spontaneous diminution of virulence in a culture which he had left for some weeks at room temperature in contact with the air. This culture, on inoculation into fowls, proved harmless. When these fowls were re-inoculated shortly afterwards with a virulent culture, instead of dying of hæmorrhagic septicæmia they recovered rapidly after a brief indisposition.

The vaccines used at present for the prophylaxis of pasteurellosis are made chiefly from strains attenuated by heat or chemical agents. In some instances their use appears to have yielded satisfactory results (Brimhall and Wilson 1900, Chamberland and Jouan 1906, Hutyra and Marek 1912, Magnusson 1914). The work of Priestley (1936) seems to show the importance of using a vaccine prepared from virulent capsulated bacilli. To avoid destroying the antigenic properties of the capsule the organisms should be killed by heating at 56° C. for not more than 30 minutes. Immune sera prepared by injection of horses with living organisms or with body exudates are sometimes employed therapeutically (Schirop 1908). The most promising method appears to be combined active and passive immunization.

According to Cardoso, Reis and Nobuga (1939), sulphanilamide has a protective effect in chickens experimentally inoculated with *Pasteurella*.

Other Diseases Associated with Pasteurella or Pasteurella-like Organisms.

Pasteurella is a common secondary invader in many diseases of animals. It has been found especially in swine fever, in which it gives rise to severe pulmonary complications, in influenza of horses (Lignières 1901), and occasionally in canine distemper (Lignières 1901), in roup (Bushnell 1923) and diphtheria of fowls (Chamberland and Jouan 1906).

The so-called " de Aar disease," described by Pirie (1929) and Mitchell (1930), affecting the veld rodents of South Africa, was probably due to a genuine *Pasteurella* infection. On the other hand the contagious pneumonia of sheep, recorded by Dungal (1931) in Iceland, was caused by an organism differing in several respects from *Past. septica* (see Chapter 32), as was also the pyogenic disease of sheep described by Jowett (1931).

A number of outbreaks in fowls (Higgins 1898), geese (Frosch and Bierbaum 1909), rabbits (Kraus 1897*a*, Kurila 1909, van Saceghem 1922), and guinea-pigs (Busson 1921) have been ascribed to infection with *Pasteurella*, which, from the records, were probably due to other organisms. There is still a disposition to treat the *Pasteurella* group as a convenient refuge for any small, Gram-negative bacillus whose identity has not been accurately ascertained. A genuine epizootic among guinea-pigs, however, has been described by Wright (1936).

PSEUDOTUBERCULOSIS

There are three or four diseases of animals known as pseudotuberculosis, which are caused by different organisms.

Pseudotuberculosis of Rodents.—In 1883 Malassez and Vignal described a disease in a guinea-pig that had been used for experimental inoculation, which was characterized by the presence of nodules histologically similar to those of tuberculosis, but containing zooglœal masses of coccoid bacilli. To this disease they gave the name zooglœal tuberculosis. A similar organism was found by Dor (1888), Charrin and Roger (1888), Nocard (1889), and Zagari (1890) in guinea-pigs dying either naturally or after inoculation with pathological material. Preisz (1894), who made a comparative study of these organisms, concluded that they were identical with a bacillus described by Pfeiffer in 1890 under the name *B. pseudotuberculosis rodentium*, now known as *Past. pseudotuberculosis*.

The disease attacks guinea-pigs chiefly, and to a less extent rats. In Germany cats appear to be not infrequently infected (Pallaske and Meyn 1932). Sporadic cases of the disease are observed in mice, rabbits, hares, pigeons, turkeys, and canaries (Pallaske 1933). Infection appears to occur by the alimentary tract. Post mortem in guinea-pigs, there are rounded greyish-white spherical nodules up to 2 or 3 mm. in diameter, distributed chiefly in the spleen, liver, and lungs. The nodules are well defined, often protrude from the surface, and on section appear creamy or caseous. Enlarged caseous mesenteric glands are generally present. Microscopically the lesions contain large numbers of short, coccoid or ovoid, bipolar-stained Gram-negative bacilli, which can be readily cultivated on agar (see Chapter 32). The disease can be reproduced experimentally in large numbers of different animals and birds (see Pallaske 1933). On injection into guinea-pigs, rabbits, rats, or mice the bacilli prove fatal in 1 to 3 weeks, depending on the dose used and the site of injection. At necropsy after intramuscular inoculation there is a caseous local lesion, enlargement of the regional lymphatic glands, and nodules in the spleen, liver, and lungs. Microscopically, the bacilli are numerous in the local lesion and glands.

Pseudotuberculosis of Mice.—This disease is due to a short Gram-positive diphtheroid bacillus, *C. murium*, described independently by Bongert (1901) and Kutscher (1894), (see Chapter 17).

Pseudotuberculosis of Sheep.—This disease has been reported in Europe, America, and Australia. It is caused by a short, non-motile, Gram-positive diphtheroid organism, known as the Preisz-Nocard bacillus or *C. ovis* (Preisz 1894). The same organism appears to be responsible for ulcerative lymphangitis of horses and perhaps for contagious acne of horses (Hutyra and Marek 1912) (see Chapter 17).

Pseudotuberculosis of Pigs.—Both Chaussé (1916) and Velu (1922) describe a disease of pigs under the name of pseudotuberculosis. The peri-pharyngeal lymph nodes appear to be chiefly affected, and the caseous lesions produced resemble those of true tuberculosis very closely. Visceral lesions may or may not be present. The cause of the disease is unknown.

Pseudotuberculosis of Man.—Topping, Watts and Lillie (1938) record a case of human infection with *Pasteurella pseudotuberculosis*. It is said to be the fifth authentic report in medical literature. Topping's case was characterized by an influenzal type of onset, fever, abdominal tenderness, neck rigidity, and later jaundice. Death occurred after 16 days. At autopsy the liver was found to be enlarged and studded with necrotic foci 1–10 mm. in diameter; the spleen was likewise enlarged and showed a diffuse lymphoid hyperplasia. An organism was isolated from the blood before death and from the liver at post mortem which was regarded as being identical with *Past. pseudotuberculosis*. Since, however, no adequate serological or pathogenicity tests appear to have been made, its exact identity must remain in doubt.

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CHAPTER 74

ACUTE RESPIRATORY INFECTIONS

One at least of the diseases dealt with in this chapter—epidemic influenza—would be more correctly placed among the virus diseases, and it is highly probable that the same applies to the common cold. Both these infections are, however, so closely connected from the clinical point of view with other respiratory diseases that it is convenient to take them out of their proper order. On the other hand, the virus pneumonias are better treated in Chapter 85, since the various agents supposed to be concerned in their causation must be discussed in relation to the lymphogranuloma-psittacosis group of filtrable viruses.

The Common Cold

Studies that have been recorded in recent years, and particularly those of Dochez and his colleagues (Shibley, Mills and Dochez 1929, Dochez, Shibley and Mills 1930, Dochez, Mills and Kneeland 1931*a*, *b*, 1932, 1933, 1936) have greatly strengthened the view advanced by Foster (1917) that the infectious common cold is a virus disease (see also Long *et al.* 1931, Powell and Clowes 1931).

Since we have as yet no satisfactory evidence that any of the ordinary laboratory animals are susceptible to infection with this virus, resort has been made to the higher apes, or to human volunteers; and this has seriously limited the scope of the experiments. One of the great difficulties that has to be overcome in work of this kind is to eliminate the risk of natural contact infection.

Dochez, Shibley and Mills (1930) recorded the transmission of the common cold to anthropoid apes, and to human volunteers, by the intranasal instillation of filtered washings from cases of the disease. Long and others (1931), using human volunteers, succeeded in several instances in inducing an acute cold with filtered nasal washings, and in transmitting it from one subject to another in series. Dochez, Mills and Kneeland (1931*a*, *b*) recorded the cultivation of the virus in Tyrode solution containing minced chick embryo, and the production of a cold in two of three volunteers with a fifteenth subculture (see also Dochez, Mills and Kneeland 1936); and Powell and Clowes (1931) have recorded similar findings.

It is probable that many points in the problem of the ætiology of the common cold—the existence of one or more types of virus, the relation of the cold virus to the influenza virus, and so on—will await the discovery of a more convenient experimental animal than man, or the anthropoid ape. In the meantime we are justified in accepting the view that the infectious common cold is a virus disease, at least as the best working hypothesis; and, for this reason, earlier observations that sought to trace a causal relationship between various bacterial inhabitants of the nasopharynx and this particular disease have become of minor interest, and may be briefly summarized.

The available records are indeed by no means consistent (see Williams, Nevin and Gurley 1921, Shibley, Hanger and Dochez 1926, Noble, Fisher and Brainard 1928, Shibley, Mills and Dochez 1929, Burky and Smillie 1929). Taking them as a whole it may fairly be said that they fail to establish any case for a consistent significant change in the bacterial flora of the nasopharynx in association with the onset of an acute cold.

In an investigation undertaken in Manchester during the period 1926–28 (see Report 1930) a nasopharyngeal swab was taken each month from 100 persons who volunteered to assist in the inquiry. Additional swabbings were taken from these persons whenever they contracted a cold. Data were thus available for a considerable number of swabbings, taken from the same persons at times when they were suffering from a cold, and at other times when they were not. The figures referring to the frequency of *H. influenzæ*, hæmolytic streptococci and the pneumococcus, are set out in Table 157. They refer only to those persons who contracted a cold during the period of observation ; and they do not include the results obtained in cases which, on clinical grounds, were diagnosed as influenza.

TABLE 157

	With Colds (110 Swabs).		Without Colds (1,731 Swabs).	
	Number +.	Per cent.+.	Number +.	Per cent. +.
H. influenzæ	52	47·27	984	56·85
Hæmolytic streptococci .	19	17·27	229	13·23
Pneumococci	30	27·27	446	25·77

It will be noted that, while pneumococci and hæmolytic streptococci were slightly more frequent in those that had colds, than in those that had not, the difference is slight and insignificant ; *H. influenzæ* was more frequently isolated at times when no sign of a cold was present. It should be added that most of these colds were trivial ; and it is probable that the factors of type and severity of infection are largely responsible for the divergent findings recorded by different workers. The " common cold " is, in fact, a disease that is impossible to define on clinical grounds. In a later investigation in London (Straker, Hill and Lovell 1939) no difference was found in the incidence of pneumococci, hæmolytic streptococci, and Pfeiffer's influenza bacilli between normal persons and patients suffering from a cold. Pneumococci and influenza bacilli, however, tended to be slightly more frequent a week after the onset of a cold than at the beginning. It is probable that these organisms are often responsible for muco-purulent inflammation of the nasal mucosa and the adjacent sinuses. In the particular case of the influenza bacillus, Dochez, Mills and Kneeland (1932) have noted the replacement of the ordinary " rough " type by the " smooth " virulent type of Pittman (see Chapter 33) following the induction of acute colds in apes with a filtrable virus.

Prophylactic Immunization against the Common Cold.

No virus-containing preparation is as yet available for active immunization. In view of what has been said above we should not expect that the injection of a bacterial vaccine, containing pneumococci, influenza bacilli, hæmolytic streptococci and so on, would have any effect on the incidence of colds, since the causal organism is not included in it. There is scarcely more reason to suppose that it would in any way diminish the severity of colds or lessen the chances of secondary infection, since the bacterial species of which it is composed are antigenically highly heterogeneous, and only a few arbitrarily selected types of each species can be included. The results of adequate clinical trials are in accord with immunological expectations.

Von Sholly and Park (1921), using a mixed vaccine of the ordinary type, inoculated 1.327 persons and kept them under observation, together with 3,025 uninoculated controls, from September 1919 to May 1920; during this period 57·7 per cent. of the inoculated and 38·2 per cent. of the uninoculated developed colds. Jordan and Sharp (1921) observed 448 inoculated persons and 461 uninoculated controls from November 1919 to June 1920; 54·9 per cent. of the inoculated and 51·6 per cent. of the uninoculated developed colds. Ferguson, Davey and Topley (1927) observed 138 inoculated persons and 148 uninoculated controls from November 1924 to May 1925; 78·3 per cent. of the inoculated and 70·3 per cent. of the uninoculated developed colds. Brown (1932) observed 80 inoculated persons and 82 uninoculated controls from November 1929 to June 1930; the inoculated contracted 1·85 colds per person during this period, the uninoculated 2·17. Further mass immunization trials with vaccine given subcutaneously, orally or intranasally have been recorded by other workers (Diehl, Baker and Cowan 1938, Siegel *et al.* 1943, McGee *et al.* 1944, Cowan and Diehl 1944, Hague 1944—see also Report 1944*c*)—likewise with negative results. It is most unfortunate that countenance should be given to the use of a vaccine so clearly devoid of protective power, and all those concerned with public health should set their faces resolutely against a practice which, though financially profitable for the makers of the vaccine, is without any scientific support.

With very few exceptions, the results of properly conducted trials are equally unfavourable to the suggestion that immunization with an " anticatarrhal " vaccine diminishes the severity of colds, or lessens the frequency of secondary infections.

It may be added for the sake of historical interest that the preparation *patulin*, a product of the mould *Penicillium patulum*, which was introduced for the treatment of colds (see Raistrick 1943, Gye 1943, Hopkins 1943), was found, when submitted to a carefully controlled trial, to be without therapeutic effect (Report 1944*a*).

Epidemic Influenza

The recent solution of the problem of the ætiology of influenza by Laidlaw and his colleagues (see Smith, Andrewes and Laidlaw 1933) will certainly stand as one of the important landmarks in the history of preventive medicine.

This disease has presented the epidemiologist with one of his most fascinating and provoking problems. It has a long and puzzling history; and, within recent years, it has been the reigning killing disease of adult life; nor is there any likelihood that its reign will end apart from some effective method of prophylaxis. The pandemic of 1918–19 was one of the great pestilences of mankind, destroying more lives in a few months than did the Great War in 4 years. Those who are interested in that pandemic and in the lessons to be learned from it may be referred to the report issued by the Ministry of Health (Report 1920). We may note that influenza is one of those diseases that seems to confer only a transitory immunity, in the sense that repeated attacks are common. We may also note that its epidemiological study has, up to the present time, been rendered unusually difficult by the lack of precision in its clinical diagnosis. There is little difficulty in telling when we are faced with an *epidemic* of influenza; but sporadic cases, and even minor outbreaks, of acute febrile diseases are very apt to receive this particular label without any very definite reason. The clinical syndrome that justifies a diagnosis of influenza is, indeed, singularly difficult to define (see Stuart-Harris *et al.* 1938). It is probable that, with the knowledge we now possess, the clinician and bacteriologist between them will be able to place this problem on a far more satisfactory footing.

The Ætiology of Influenza.

In view of our present knowledge the long controversy as to whether epidemic influenza was, or was not, caused by *H. influenzæ*, described by Pfeiffer in 1892, has lost all but historical interest.

Those interested may be referred to the monograph by Kristensen (1922). We may summarize the extensive bacteriological literature by noting that, while there is ample evidence that the influenza bacillus is very frequently present in the nasopharynx of those suffering from the disease (see for instance Pritchett and Stillman 1919, McClelland 1919, Park 1919, Duval and Harris 1919, Schorer 1919, Medalia 1919, Dick and Murray 1919, McIntosh 1922), and though its distribution in the tissues, as revealed by post-mortem examination, makes it clear that it often plays a part in the disease as it appears in man (see Fildes and McIntosh 1920, McIntosh 1922), its curious absence in certain epidemics, particularly in the summer of 1918, had raised serious doubts as to its primary ætiological rôle before the view that influenza was a virus disease had been clearly established (see Mandelbaum 1918, Selter 1918, Messerschmidt *et al.* 1919, Leichtentritt 1918, Kristensen 1922).

The virus theory of influenza was, of course, held quite widely as a possible working hypothesis, before the problem was attacked by Laidlaw and his colleagues, and there had been occasional reports of the transmission of an influenza-like disease to apes or to human volunteers with filtered washings from influenzal patients; but none of these experiments was on such a scale as to establish any definite conclusion.

The most important contribution to the influenza problem, prior to the original report of Smith, Andrewes and Laidlaw (1933) was the work of Shope on influenza in swine, and this will be considered in a later section.

In view of their great importance the observations of Laidlaw and his colleagues must be discussed in some detail.

In their original communication Smith, Andrewes and Laidlaw (1933) showed that a characteristic febrile disease could be induced in ferrets by the intranasal instillation of filtered nasal washings from a human case of epidemic influenza. This disease was transmissible in series, from ferret to ferret, either by natural contact or by the intranasal instillation of filtered material. Ferrets that had recently recovered from the disease were resistant to re-infection, and their serum contained protective antibodies in high concentration. In this report it was also noted that Shope's swine influenza virus produced in ferrets a disease indistinguishable from that produced by the human virus, and that there appeared to be an immunological relationship between the two types. These observations were later confirmed and extended (see, also, Shope 1935, Francis and Shope 1936). Shope (1934*b*), who had successfully repeated the experiments of Smith, Andrewes and Laidlaw, found that the swine influenza virus when injected intranasally into ferrets under ether anæsthesia produced pneumonic lesions in the lungs, and Andrewes, Laidlaw and Smith (1934), using a similar technique, were able to induce a fatal pneumonia in mice, with both the human and swine viruses. Francis (1934) isolated a strain of the human virus from cases of influenza in Puerto Rico, and this virus and other strains isolated later in New York and Philadelphia were found to be immunologically identical with the original English strain, and with other strains isolated in England during the winter and early spring of 1934–35 (Laidlaw 1935, Andrewes, Laidlaw and Smith 1935, Francis and Magill 1935*a*). An immunologically identical virus has since been isolated from epidemics of influenza in

Australia (Burnet 1935*a*), Alaska (Pettit, Mudd and Pepper 1936), and other parts of the world (see Mote 1941). This virus is now generally referred to as the influenza A virus. Failure to find it in some outbreaks led to the suspicion that more than one type of virus might exist. Variants of the A virus were described by Magill and Francis (1936); and later, Magill (1940) and Francis (1940*b*) independently discovered a new type, antigenically distinct, which is now known as influenza B. There is reason to believe in the existence of at least one more type, since Lennette and his colleagues (1941), in a study of 19 separate outbreaks of influenza, could find no evidence of either A or B virus in over 30 per cent. of the cases examined.

Laidlaw (1935) suggests that the swine influenza virus may represent the 1918–19 human virus which has become acclimatized to swine. This tentative hypothesis is based in part on the proved immunological relationship, but not identity, of the two viruses, in part on the fact that, while neutralizing antibodies to the human virus were found in most human sera, antibodies to the swine influenza virus were regularly present in adult human sera, but were wholly absent from the sera of a small sample of children (Andrewes, Laidlaw and Smith 1935). Similar observations have been recorded by Francis and Magill (1936), and by Shope (1936). The correctness of Laidlaw's interpretation is rendered doubtful by the finding that influenza A virus can give rise to antibodies against the swine influenza virus, and that a similar age distribution of antibodies as that found 15 years after the 1918–19 pandemic seems to exist at the present time (Horsfall 1943).

The evidence in favour of the virus causation of influenza has been summarized by Andrewes (1937). Considerable difficulty has been experienced in the reproduction of the disease in man with laboratory strains of the A type of virus. Successful results have been obtained, but only irregularly. One reason for this may be that many adults contain neutralizing antibodies in their serum. The higher the antibody titre, the greater, on the whole, is the resistance of the subject to infection, though it is clear that other factors influence the result (see Horsfall 1943). Resistance to Type B seems to be less general; and in one trial Francis and his colleagues (1944) appear to have been able to reproduce the disease in practically every subject tested.

How influenza infection is kept going from one epidemic to another is doubtful, but there is some evidence to suggest that chronic carriers may exist (Taylor and Dreguss 1940).

Properties of the Virus.—There has been some dispute over the size of influenza A virus. Elford, Andrewes and Tang (1936), using the gradocol membrane technique, found it to fall between the limits of 80 and 120 mμ; and Elford and Andrewes (1936), using the centrifugation technique, estimated it at between 87 and 99 mμ. Chambers and Henle (1943), however, concluded from centrifugation experiments that it was only 10 mμ in diameter. Stanley (1944), who studied the reason for this discrepancy, showed that the behaviour of the influenza virus in dilute solutions of electrolyte was variable, and he brought evidence to show that the real size was probably about 70 mμ. Taylor and his colleagues (1943), as the result of centrifugal separation of Virus A, confirmed Elford and Andrewes' observations, finding the mean particle size to be 77·6 mμ. This estimate is identical with that reached by Sharp and his colleagues (1944), who examined the A virus by the electron microscope, and concluded that the particles were kidney-shaped and had a mean diameter of 77·6 mμ. Lauffer and Stanley (1944), however, obtained a value by the electron microscope method (Fig. 291) of

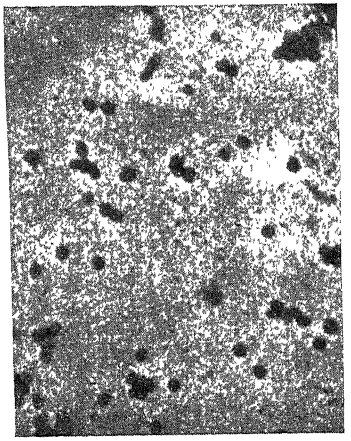


FIG. 291.—Electron micrograph of influenza virus Type A (× 17,750). (Kindly supplied by Dr. W. M. Stanley.)

115 mμ, and by the centrifugation technique of 80 mμ. They regard the discrepancy between these two findings as due to a high water content of the virus particles, and they suggest that the real value is about 100 mμ. The B virus was found by Sharp and his colleagues (1944) to be circular or ovoid in shape, and to have a mean particle diameter of 97·3 mμ. The swine influenza virus, according to Taylor and his colleagues (1944), resembles in size Virus A, being about 78–81 mμ in diameter.

The virus can be grown in tissue culture (Francis and Magill 1935*b*), and on the chorio-allantoic membrane of the developing chick embryo (Burnet 1935*b*). Though these observations were made on strains adapted to the mouse or the ferret, success was later recorded in direct culture experiments from human material. It is interesting to note that inoculation of the chick embryo with Virus A interferes with the growth on subsequent inoculation of Virus B or the swine influenza virus. Any one of these three viruses, in fact, prevents the growth of either of the other two—subject to certain time and qualitative relationships (Ziegler and Horsfall 1944, Ziegler, Lavin and Horsfall 1944) (see also Chapter 55, p. 1236).

The antigenic structure of the influenza virus is still the subject of detailed observation. The A and B viruses are antigenically distinct, but within each type there appears to be considerable heterogeneity (see Mote 1941). Variants of the A virus have been described by Magill and Francis (1936), Magill and Sugg (1944), and Friedewald (1944), and variants of the B virus by Eaton and Beck (1941) and Gordon (1942). Some of the Type A strains seem to be related more closely to the British swine influenza viruses than to the American swine influenza virus (Glover and Andrewes 1941, Hudson *et al.* 1943). According to Burnet (1942), the human virus, though not antigenically related to the Newcastle disease virus of chicks, nevertheless behaves like it in many respects. Studies have revealed the existence of two antigenic fractions in the influenza virus: (*a*) a fraction intimately associated with the virus particle and readily adsorbed by fowl red blood corpuscles, and (*b*) a soluble fraction which remains in the supernatant fluid of centrifuged suspensions, and which is not adsorbed by red corpuscles. The body antigen appears, by the complement-fixation test, to be more specific than the soluble antigen (see Lennette and Horsfall 1940, Friedewald 1943).

According to Knight and Stanley (1944), the purified virus is inactivated promptly by 5 per cent. phenol, in a week by 0·5 per cent. phenol, and in a month by 0·1 per cent. phenol. The effect of other disinfectants has been studied by Dunham and MacNeal (1944).

A most interesting property of both types of influenza virus—also shared by the Newcastle disease virus of chickens (Burnet 1942)—is their ability to agglutinate the red blood corpuscles of fowls and certain mammals (Hirst 1941, 1942, Hirst and Pickels 1942). Provided the strains are fresh and fully pathogenic, the agglutination titre runs parallel to the mouse lethal titre. Agglutination is inhibited specifically by immune serum, the degree of inhibition affording an index to the ability of the serum to neutralize virus. The test can therefore be used diagnostically for detecting the presence either of virus or of antibodies (see also Miller and Stanley 1944).

Burnet and Bull (1943) found that freshly isolated strains of Virus A agglutinated guinea-pig red corpuscles to a much higher titre than fowl corpuscles, but that after passage in series through chick embryos they agglutinated both types of corpuscles to much the same extent. Other changes likewise occurred as the result of passage, closely reminiscent of variation in bacterial cultures. Observations suggested that the degree of agglutination of the red corpuscles of a particular species of animal, including man, afforded a good index of the degree of susceptibility of that species to infection.

Diagnosis.—The laboratory diagnosis of influenza is made by demonstration of the virus in the nasopharynx, or of complement-fixing or neutralizing antibodies in the blood serum. The presence of the virus may be established by the injection of garglings or sputum, taken during the first two or three days of the disease, into the nose of a ferret. If virus is present, the animal develops a febrile illness after about 48 hours, accompanied by rhinitis. Passage of the virus through anæsthetized ferrets results in the production of pneumonia, which is often fatal. Alternatively, the nasopharyngeal washings of the patient may be injected intranasally into mice or into the amniotic sac of the developing chick embryo. Neither of these methods, however, is reliable, since many strains of virus fail to produce obvious lesions on first inoculation. Complement-fixing or neutralizing antibodies may be demonstrated in the patient's serum within about a week of the beginning of the disease. By the use of suitable control strains and sera the type of infecting virus to which neutralizing antibodies have appeared may often be determined by the intraperitoneal injection of mice. It must be remembered, however, that there is considerable antigenic variation among different strains, and clear-cut results are not always obtained (see, for example, Magill and Sugg 1944).

Immunization.—The problem of immunization against the influenza virus is clearly of primary importance. If we could induce an effective active immunity in man we should be in a position to eliminate the disease, or reduce it to negligible proportions. It is possible that passive immunization, by the injection of an antibody-containing serum, might exert some therapeutic effect in an established case; but this method, even if successful, should clearly be regarded as a second and subsidiary line of defence.

According to the observations of Smith, Andrewes and Laidlaw (1935), ferrets that have recovered from an attack of the virus disease are, for a time, completely resistant to infection. This solid immunity persists for about 3 months, and then gradually wanes. Ferrets with a waning immunity still show the presence of protective antibodies in their blood, although the nasal mucosa has become susceptible to re-infection; and, when it is re-infected, the circulating antibodies fail to protect the animal against a general febrile reaction. The position in regard to active immunization, apart from the production of an attack of the disease, is curious and interesting. The ferret is not susceptible to the injection of the living virus otherwise than by its intranasal instillation or by some other method of introduction directly into the respiratory tract. Animals inoculated subcutaneously, intradermally, intraperitoneally, intramuscularly, intratesticularly or intracerebrally show no sign of illness. Such injections, particularly if frequently repeated, lead to the appearance of protective antibodies in the circulating blood; but the appearance of these antibodies is not associated with any effective resistance to infection by the intranasal route. This clearly suggests that the local con-

ditions in the nasal mucosa are of predominating importance. This is borne out by the observations of Burnet, Lush and Jackson (1939) and of Francis (1940*a*), who have demonstrated the presence in the serous nasal secretion of normal human beings of an agent capable of neutralizing the influenza A virus. According to Francis, this substance appears to be none other than specific neutralizing antibody, but whether it is derived wholly from the serum antibody is still doubtful. So far, attempts to induce immunity by the intranasal injection of killed or inactivated virus have failed, though the direct instillation of antibody into the nose has been shown to be of greater protective value in mice than much larger quantities injected into the blood stream (see Horsfall 1943).

If a ferret that is in the stage of waning immunity is injected subcutaneously with living virus, its immunity may be reinforced, so that it becomes again completely resistant to intranasal infection. Immunization by the subcutaneous route is not therefore completely without effect; and this is also shown by the fact that the injection of living or formolized virus into normal ferrets, although it induces no effective immunity to re-infection by the intranasal route, lessens the frequency of lung lesions. A similar effect may be observed in mice. From animal experiments, therefore, it would appear that the most that has yet been accomplished on the lines of active immunization is the production of a partial immunity of a relatively low grade.

Numerous attempts have now been made in man to vaccinate against influenza (Francis and Magill 1937, Stokes *et al.* 1937, Horsfall *et al.* 1941*a*, *b*, Hirst *et al.* 1942, Burnet 1943, Henle *et al.* 1943, Francis *et al.* 1943, 1944, Report 1944). Virus inactivated by a variety of methods gives rise to the same specific antibodies as living virus. The rise in antibody titre after vaccination seems to be related to the pre-vaccination titre of existing antibodies as well as to the dose and quality of the vaccine. In general, the higher the antibody titre at the time of exposure to infection, the greater is the degree of resistance; though it is clear that the antibody titre is only one factor in determining this result. Since repeated attacks of influenza may occur under natural conditions, it is hardly to be expected that more than a partial degree of transient immunity is likely to result from vaccination. This has been strikingly demonstrated by Francis and his colleagues (1944), who were able to produce two attacks of influenza within 4 months in human subjects inoculated intranasally with the same living strain of Type B virus. The second attacks were less severe than the first, but the fact that they occurred at all showed that the increased resistance following the first attack was relatively slight. If immunity even to the same type of virus is weak and transient, then protection against numerous different antigenic variants may prove very difficult to achieve.

In practice it may be found more expedient and economical to wait till an influenza epidemic has started, to determine the type of infecting virus, and then to inoculate the population at once with the corresponding vaccine. Antibodies are known to reach a high level within two weeks of inoculation, so that this method might succeed in lowering the case morbidity, even though it was given too late to protect those smitten early in the outbreak (see Hirst, Rickard and Friedewald 1944). (For preparation of influenza virus vaccine, see Stanley 1945.)

In regard to passive immunization, it has proved possible to produce an anti-influenzal horse serum that contains neutralizing antibodies to relatively high titre (see Laidlaw, Smith, Andrewes and Dunkin 1935). This serum, when injected

intraperitoneally into mice in relatively large doses, significantly increases their resistance to the intranasal injection of virus, under light ether anæsthesia, 45 minutes later. It also leads to a reduction in percentage mortality when injected intravenously or intraperitoneally into mice 24–48 hours after the intranasal instillation of virus. Better protection can be obtained with much smaller doses injected intranasally prior to nasal infection. Apparently successful attempts to prevent the development of influenza in human beings during an epidemic by inhalation of vaporized antiserum have been recorded by Smorodinzew and his colleagues (1940), but it is as yet too early to say whether this method will prove of value in practice.

In the past many attempts have been made to induce an immunity to influenza by the injection of a vaccine containing *H. influenzæ*. Clearly, such a procedure has no scientific justification and it is sufficient to note that, here again, adequate field trials are in accord with immunological expectations (see von Sholly and Park 1921, Jordan and Sharp 1921). For recent studies and reviews on influenza, see Stuart-Harris *et al.* (1938), Mote (1941), Horsfall (1943), and Stuart-Harris (1945).

Swine Influenza

We have referred above to Shope's observations on swine influenza (see Shope 1931*a*, *b*, 1932, 1934*a*, *b*, 1935, Lewis and Shope 1931). This disease first appeared, or was first recognized, in the United States in the autumn of 1918. It occurred in epidemic form in close association with the human pandemic of influenza, and it was at once suggested that there was some causal relation between them.

Shope (1931*a*, *b*) was able to show that the swine disease, in its characteristic clinical form, was caused by the associated effects of a filtrable virus and a hæmophilic bacillus, closely resembling the human influenza bacillus, to which he gave the name of *H. influenzæ-suis* (see Chapter 33). The virus alone caused a relatively trivial infection, unassociated with the pulmonary complications of the natural epidemic disease. The bacillus alone caused no detectable illness. As in the infection of ferrets with human influenza virus, the swine virus must be injected intranasally in order to produce any symptoms of disease ; injected intramuscularly it has no effect, either in inducing the mild virus disease or in rendering the animal sensitive to the intranasal instillation of *H. influenzæ-suis* (Shope 1932). The intramuscular injection of the virus does, however, render swine immune both to the mild virus disease and to the severe disease caused by the associated effect of the virus and the hæmophilic bacillus. Injections of *H. influenzæ-suis*, on the other hand, induce no immunity either to the virus or to the combined action of the virus and bacillus (Shope 1932). Inoculated into the developing chick embryo, the virus, in association with *H. influenzæ-suis*, gives rise to a mortality several times that caused by either agent alone. The two together cause destruction of the embryo lungs (Bang 1943).

As noted above, Shope (1934*b*) has shown that the swine virus, acting alone, will produce in ferrets a disease that is indistinguishable from that produced by the human influenza virus. He has also (Shope 1935) transmitted the virus to mice. The immunological relationship of the human and swine viruses, and its possible significance, have been discussed above. Antigenically, the American swine influenza virus strains appear to be homogeneous, whereas the British strains differ not only from the American strains, but to some extent from each other. They appear to occupy a position in some ways intermediate between the American

swine strains and the human Type A strains of influenza virus (Glover and Andrewes 1941).

The epidemiology of swine influenza has been illuminated by the studies of Shope (1941, 1943*a*, *b*). The disease tends to break out more or less simultaneously among separated herds of swine during the last three months of the year, particularly after some change in the weather, suggesting that the swine are already infected with the virus, but that some circumstance is needed to activate it. Shope has brought evidence to show that swine lungworms—*Metastrongylus elongatus* and *Choerostrongylus pudendotectus*—play an important part in the propagation of the disease. Lungworms taken from sick or from convalescent swine have been found to carry the virus, though in a masked form. The infected ova are coughed up by the pig and excreted in its fæces. They are swallowed by earthworms, in which they pass through three larval stages. The third stage is infective for swine. If the earthworm is then eaten by a pig, the larvæ penetrate the intestinal mucosa and migrate to the respiratory tract by way of the lymphatics and blood stream. After two further developmental stages, they finally become adult lungworms and occupy the bronchioles at the base of the diaphragmatic lobes. If infected earthworms are fed experimentally to swine, nothing happens; but if the animals are given multiple inoculations intramuscularly of living *H. influenzæ-suis*, some of them develop typical influenza—provided the experiment is carried out during the winter. Both the lungworms and the earthworms may remain infected for several months. The infected earthworms are probably eaten by swine at the time of the late spring and early autumn rains, when the worms come to the surface of the soil. The pig becomes infected in this way with the virus—still in the masked form. If at the time *H. influenzæ-suis* happens to be present, then it requires only some circumstance, such as a climatic or physical change—perhaps some weeks or months later—to activate the virus, allow it to penetrate the cells of the respiratory tract, and give rise to typical influenza.

Influenza in other Animals.

There are several other animal diseases, occurring in sporadic or epidemic form, to which the label "influenza" has been attached; "horse influenza" is an example. In the particular instance of the *horse* disease there is suggestive, if not conclusive, evidence that it is caused by a filtrable virus; and it is altogether probable that we shall find that several animal diseases are in fact due to infection with a virus of the influenzal type (see Discussion 1935). There is evidence, for example, that the disease of *calves* variously referred to as infectious pneumonia, calf influenzal pneumonia, and perhaps sometimes as scours, is due to a virus infection. The disease is widespread in Great Britain and in certain parts of the United States. It is characterized by fever, pneumonia, and in the later stages diarrhœa. The case mortality may be as high as 50 or 60 per cent. Animals killed at the height of the disease show a catarrhal enteritis and a bronchopneumonia. The disease can be transmitted by ground-up lung suspensions that have been filtered through a Berkefeld N candle or a Seitz EK disc (Lamont and Kerr 1939, Baker 1943). Baker has been able to transmit the infection to mice, and to reinfect calves with the pneumonic lungs of these animals. Calves that have recovered contain neutralizing antibodies in the blood serum.

Whooping Cough

H. pertussis, the causative organism of this disease, was first isolated and described by Bordet and Gengou (1906) (see Chapter 33). Its ætiological rôle is shown by the following observations. It can be isolated in the great majority

of cases during the first 2 weeks of the disease by the cough-plate or post-nasal swab method, or from the lungs at autopsy. It has seldom been demonstrated in normal persons, possibly because of the difficulty of detecting it when present in only small numbers. In the later stages of the disease and during convalescence specific antibodies make their appearance in the blood.

It may be added that Macdonald and Macdonald (1933) record the experimental production of whooping cough in two children with a culture of *H. pertussis*, and that Shibley and Hoelscher (1934) have reported the successful reproduction of the disease in the chimpanzee. A mild disease can be reproduced in rhesus monkeys (*M. mulatta*) by intranasal or intratracheal inoculation, and the organisms can be recovered from the lungs if the animals are killed within the following two weeks (North *et al.* 1940). The infections produced in ordinary laboratory animals, which bear no resemblance to the disease in man, are described in Chapter 33.

Whooping cough is mainly a disease of infancy and early childhood. Half of the deaths occur during the first year of life. Since the middle of last century the mortality of the disease has fallen greatly both in this country and in the United States (see Stocks 1942, Dauer 1943). The incubation period varies, but is round about 10 days. The duration of the catarrhal stage is usually about 11 days. The patient is infectious chiefly during this stage and to a less extent during the first week of the paroxysmal stage (see Lawson 1933).

Diagnosis.

The clinical diagnosis of whooping cough is often difficult and confirmation may be sought from the laboratory. During the catarrhal stage there is usually a leucopenia, but this changes during the paroxysmal stage to a leucocytosis with an absolute and relative lymphocytosis. The constancy of these findings is under dispute, but blood changes may be of help in cases seen too late for cultural methods to be of value.

Cultural.—As has been stated, *H. pertussis* can almost always be isolated from the sputum during the early stages of the disease (see Wollstein 1909, Chievitz and Meyer 1916). For several years the cough-plate method has been extensively used for diagnostic purposes, and has given satisfactory results. The patient is induced to cough directly on to a plate of Bordet-Gengou medium, and this is examined for the characteristic colonies after 48–72-hours' incubation (see Chapter 33).

Among reports on results obtained by this method are the following. Madsen (1925), in 914 cases, records 75 per cent. of positive isolations during the initial catarrhal stage, 61 per cent. during the 2nd week, 45 per cent. during the 3rd, 40·5 per cent. during the 4th, and 9 per cent. during the 5th. Sauer (1933*c*), in 400 cases, records 88 per cent. of positive isolations in the early catarrhal stage, 64 per cent. in the paroxysmal stage, and none in the late stages. Gardner and Leslie (1932), in 47 cases, record 67–75 per cent. of positive results in the first 3 weeks, 25 per cent. in the 4th and none thereafter. Kendrick and Eldering (1934), in 207 cases, record 78–84 per cent. of positive results during the first 3 weeks, 39 per cent. in the 4th and none in the 6th (see also Sugare and McLeod 1929, Sauer and Hambrecht 1930, Kristensen 1933, Straker and Westwater 1937, Donald 1938). It may be added that Kristensen (1933) records his failure to isolate *H. pertussis* from 500 healthy persons, and from 202 persons suffering from other respiratory diseases. Among 301 contacts, *H. pertussis* was isolated in 9 instances, and all these 9 persons subsequently developed whooping cough. Kendrick and Eldering (1934) and Wilcox

(1934) also record their failure to isolate the bacillus from any condition other than whooping cough.

More recently the post-nasal or nasopharyngeal swab has been introduced to avoid some of the inconveniences of the cough-plate method. Cultures from the swab are liable to be overgrown by other organisms, but if the swab is streaked on to a Bordet-Gengou plate over which a few drops of a suitable strength of penicillin have been spread, a high proportion of successful isolations may be achieved. Since Maclean (1937) first described this method, numerous observers have confirmed its value.

American workers (Bradford and Slavin 1940, Brooks and Bradford 1942, Miller *et al.* 1943) have used a very fine swab passed through the anterior nares till it reached the pharynx. In this country a bent swab passed through the mouth and up behind the soft palate has been preferred (Cruickshank 1944). Our own experience favours the latter method. In comparisons made by these different workers between the cough-plate and the swab methods, the results were consistently better when swabs were used, though a combination of the two methods gave the highest proportion of positive results.

Serological Tests.—The demonstration that specific complement-fixing antibodies appeared in the blood was first made by Bordet and Gengou (1906, 1907). The antigen employed is a suspension of *H. pertussis*.

These observations were confirmed by Winholt (1915) and by Chievitz and Meyer (1916), who showed that the reaction first becomes positive in the 3rd or 4th week, the proportion of cases reacting positively increasing up to the 8th or 10th week. The reaction usually becomes negative again in the course of a few months. Similar observations have since been reported by many observers (see Madsen 1925, Gundel and Schlüter 1933*a*, *b*, Donald 1938). There is general agreement that a small proportion of cases fail to develop a positive reaction at any stage in the disease.

Besides complement-fixing bodies, agglutinins appear in the serum. Their presence is best detected by a slide technique using living cultures of Phase I organisms. Evans and Maitland (1939), who compared the two methods, obtained a higher proportion of positive results by agglutination than by complement fixation.

A skin test has been recommended by Strean (1940) and by Smolens and Mudd (1943) for the diagnosis of susceptibility to infection (see also Strean and Grant 1940). The toxin is prepared by aqueous extraction of the frozen and thawed organisms or by acid extraction of a fresh culture. The value of this test is still in doubt.

It will be seen, therefore, that attempted cultivation by the cough-plate or the nasopharyngeal swab method is the diagnostic method of choice during the early stage of the disease, and before the characteristic paroxysmal cough has developed. It is at this stage that a bacteriological method of diagnosis is most helpful. In those cases in which the diagnosis remains in doubt for 3–4 weeks both cultural and serological tests may be employed. Where a laboratory diagnostic method is sought in a case of more than 4 weeks' standing the complement-fixation or agglutination test should be used.

Prophylactic Vaccination against Whooping Cough.

Reference to Chapter 33 will show that *H. pertussis* is antigenically of a single type. The conditions are, therefore, particularly favourable for active immunization with a vaccine of this organism. It has been noted, however, in the same chapter that this organism is very liable to undergo variation in the laboratory, and that the variant strains are of low virulence for laboratory animals and fail

to produce an effective immunity (see Leslie and Gardner 1931). It is therefore of the greatest importance that strains used for immunization should be fully virulent (Phase I of Leslie and Gardner); and the fact that this necessity has not always been allowed for must be borne in mind in assessing the published records of immunization in the field. Among the large-scale trials that have so far been reported are the following:

Madsen (1933) records trials made in the Faroe Islands during an epidemic in 1923–24, and in another epidemic in 1929. In the first epidemic the experience of 2,094 vaccinated subjects was compared with that of 627 unvaccinated controls. The incidence of the disease was the same in the vaccinated and unvaccinated, but it was milder and the death rate was lower among the vaccinated. In the preparation of the vaccine used in this trial several recent strains were used. In the epidemic of 1929, the vaccinated numbered 1,832, the unvaccinated 466. The experience in this epidemic was more favourable, 458 of the 1,832 vaccinated subjects escaped attack, as compared with 8 of the 466 unvaccinated controls. The vaccine used in this trial was prepared from a freshly isolated strain of *H. pertussis*. In the second trial, as in the first, the disease was less severe and the deaths proportionately fewer among the vaccinated than among the unvaccinated. Taking both epidemics together, the mortality among the vaccinated was 0·15 per cent., as compared with 2·4 per cent. among the unvaccinated. These trials suggested that prophylactic immunization with an *H. pertussis* vaccine is only moderately effective in preventing the disease, but is of very real value in decreasing severity and mortality.

Extensive trials have been carried out by numerous workers in the United States and Canada (see Sauer 1933*a*, *b*, *c*, 1935, Kendrick and Eldering 1936, Siegel and Goldberger 1937, Doull *et al.* 1939, Rambar *et al.* 1941, Bell 1941, Daughtry-Denmark 1942, Perkins *et al.* 1942). With the exception of the series recorded by Doull and his colleagues, the results were uniformly favourable. Success has also been reported by Oláh (1942) in Hungary and by Dungal, Thoroddsen and Ágústsson (1944) in Iceland.

Before accepting these findings, it is necessary to point out that in some of the observations recorded there was no control group, and that in others the method of selecting the controls was not above criticism. In mass immunization experiments too much care cannot be taken to ensure that the treated and the control subjects are as similar as possible in every respect. The fallacy of offering immunization to a given group of subjects, inoculating those who accept and treating the remainder as controls, has already been pointed out in relation to B.C.G. vaccination (p. 1339). It is particularly difficult in clinic practice to be sure that children in the two groups are exposed to equal risks of infection. The most reliable method, therefore, is to inoculate alternate children in a residential nursery or similar institution, in which all the children are mixed together and in which infection, if it is introduced, will spread to all alike. A small investigation made under these conditions by McFarlan and Topley (1943) in a residential nursery in Berkshire revealed no difference in either the incidence or the severity of the disease between the vaccinated and the control groups. A similar completely negative result was obtained by McFarlan, Topley and Fisher (1945) in a carefully controlled trial in clinics and day nurseries at Oxford.

What is the explanation of these discrepancies? Several factors have to be considered, such as the mode of preparation of the vaccine, the size, number and spacing of the doses, and perhaps most important of all the perfect equalization in every respect of the groups of inoculated and control children. This is not the place to enter into a critical analysis of these factors; suffice it to say that they differed in practically every one of the trials referred to. We feel therefore

that, though strong presumptive evidence has been brought forward by American workers for the value of inoculation in the prophylaxis of whooping cough, the case is still far from proven. Further trials under more stringent conditions must be carried out before the efficacy of this method can be accepted, and before it is introduced as a routine measure into child welfare practice.

Serum Treatment.—Since 50 per cent. of deaths from whooping cough occur before the age of one, and since the formation of antibodies during the first few months of life is poorer than it is later (see Baumgartner 1933–34, 1937), it is clear that, even if a satisfactory vaccine was available, it would be of little help in the protection of very young infants. For these a therapeutic serum is required. Mention has already been made in Chapter 33 of the separation of a toxin from *H. pertussis*. Evans (1940) has shown that by the injection of toxin into rabbits an antitoxic serum can be produced, capable of neutralizing the necrotic effect of toxin introduced into the skin. How far such a serum is likely to be of value in human beings is at present doubtful. Bullowa and his colleagues (1942) found that antitoxic serum prepared in rabbits had no therapeutic activity in children, but if given prophylactically early in the incubation period of the disease, it appeared to protect a high proportion of them against whooping cough. Strean (1944), on the other hand, has claimed valuable therapeutic activity for the serum (see also Strean, Lapointe and Dechene 1941). Further work will obviously be required to establish the value of serum in the prevention and cure of whooping cough. It may be added that the sulphonamide drugs have already proved their worth in the treatment of secondary broncho-pneumonia, which is so often responsible for a fatal outcome in infants and young children.

Secondary Broncho-Pneumonia

Influenza, whooping cough, measles, and to a less extent certain other specific infective diseases, are peculiarly liable to be associated with a secondary infection of the lungs ; and it is this fact which is answerable for the high mortality associated with such diseases. The case mortality during an epidemic of influenza, for instance, is almost entirely determined by the frequency of pulmonary complications. Without entering into the pathology of these pulmonary lesions we may note that the distribution is usually of the broncho-pneumonic type ; and that the pneumonias associated with influenza are frequently characterized by certain features, which serve to distinguish them from those which follow measles or whooping cough. Thus, in cases of influenzal pneumonia, the lesions of the bronchi are often unusually severe ; hæmorrhages into the alveoli, into the bronchi, or around the bronchioles, are frequently encountered ; and localized areas of necrosis, or suppuration, are not uncommon (see Opie *et al.* 1921).

The bacteriology of secondary broncho-pneumonia in general, and of influenzal pneumonia in particular, differs from that of primary lobar pneumonia in the fact that the pneumococcus plays a far less predominant rôle ; and that, among the pneumococcal cases, the commoner " infective " serological types (see next section) are found to be responsible for only a small minority of the infections. In influenzal pneumonia, *H. influenzæ* may play a dominant rôle. Hæmolytic streptococci, which play a negligible rôle in primary lobar pneumonia, frequently invade the lung during an influenzal attack, and give rise to a particularly fatal type of infection. In whooping cough it is probable that *H. pertussis* itself plays a large, but not an exclusive, part in the production of lung lesions.

The species of bacteria isolated from the sputum, blood, or empyema fluid during life, and from the lungs or other tissues at autopsy, vary from place to place and from time to time. There is definite evidence, in the records, of a secondary local prevalence of a particular bacterial species, imposed on the primary prevalence of influenza, or of measles; so that one observer may record a high incidence of streptococcal pneumonia, another a considerable series of secondary pneumococcal infections, and a third may note the occurrence of numerous pulmonary infections due to *Staph. aureus* (see for example Smith 1935, Finland, Peterson and Strauss 1942). Among the less frequent infective agents we may note *Bact. friedländeri*, capable of producing an acute, suppurative and usually fatal pneumonia (see Solomon, 1937, 1940, Hyde and Hyde 1943) and streptococci of the *Str. viridans* type (Solomon and Kalkstein 1943, Thomas 1943). The figures recorded by Dwinell (1919), in 69 autopsies on fatal cases of influenzal pneumonia, may be regarded as typical of an outbreak in which hæmolytic streptococci are playing a predominant part. The frequency of the various bacterial species in this series of cases was as follows.

		Per cent.
Hæmolytic streptococci	41 cases	= 59·4
Pneumococcus (all types)	8 „	= 11·6
Non-hæmolytic streptococci	17 „	= 24·6
Staphylococci	13 „	= 18·9
H. influenzæ	34 „	= 49·3

The predominance of pneumococcal types other than I, II, and III in any outbreak of influenzal pneumonia in which *Str. pneumoniæ* is the principal secondary agent is illustrated by the following figures, giving the type of pneumococcus isolated from 105 cases of influenzal pneumonia (Opie *et al.* 1921).

	Per cent.
Type I	7·6
Type II	21·9
Type III	5·7
Other Types	64·8

In many infections by the organisms mentioned in this section, the disease may, on both clinical and pathological grounds, be classified as a primary pneumonia. In others, the distinction between primary and secondary pneumonia is hard to make and, from many points of view, may not be worth making. Nevertheless, the term "secondary pneumonia" at least serves to remind us of the patient's predisposition to bacterial respiratory infections in diseases like measles and influenza; and from the foregoing it is clear that pneumococci, influenza bacilli, hæmolytic streptococci and staphylococci are the chief offenders in taking advantage of this predisposition, together accounting for the great majority of fatal infections.

The fact that these secondary pneumonic infections play a large part in determining the case-mortality rate in any outbreak of influenza, measles or whooping cough, raises a point of the first importance in regard to the effective control of these diseases. It is probable that the secondary bacterial invader responsible for the fatal issue is in many cases leading a vegetative existence in the upper part of the respiratory tract of the host, at the time when the primary infection is contracted. In other cases the host may receive the secondary invader

from the same source, and at the same time, as the primary infecting agent. There is, however, little doubt that a considerable proportion of fatal secondary infections result from the implantation, on a case of primary influenza or measles, of a virulent streptococcus or pneumococcus; and the records contain instances of waves of secondary infection, due to a particular bacterial species, passing through hospital wards, or concentration camps (see Opie *et al.* 1921). The conditions which, in the aggregate, make up the environmental factor known as "hospitalization," play the same part in secondary respiratory disease, as they play in the case of surgical or puerperal sepsis. The patient with influenza or measles should be regarded as peculiarly liable to acute respiratory infection, and should be carefully guarded from secondary infection. There is, for instance, ample evidence that overcrowding in hospital wards, or in military camps, may seriously increase the mortality during an outbreak of influenza or of measles (see Memorandum 1944).

There seems little hope that any form of prophylactic immunization, apart from immunization against the primary disease, will reduce the liability to such secondary infections. As will be obvious, we are faced with a multiplicity of heterogeneous bacterial antigens, and it seems scarcely reasonable to expect that we can ever induce immunity to all the bacterial species and types, to which a person with some primary predisposing infection may become a prey. The same heterogeneity of infecting agents severely limits the opportunities for the use of specific serum therapy.

Both the sulphonamides and penicillin are reported to be effective in the control of broncho-pneumonia due to staphylococci and streptococci. The sulphonamides are less effective against *H. influenzæ* and *Bact. friedländeri*, though some success is claimed for them.

Primary Pneumococcal Pneumonia

We need not here consider the clinical or pathological aspects of pneumonia as it occurs in man, nor discuss the vexed question of the classification of acute inflammatory lesions of the lungs. Excluding the broncho-pneumonias of young children, and the secondary pneumonias to which we have already referred, there remain a group of cases in which a severe pyrexial illness, with sudden onset and a tendency towards recovery by crisis in non-fatal cases, is associated with signs and symptoms of pulmonary involvement; and in which bacteræmia is frequently present during the initial phase of infection, and is a constant feature in severe and fatal cases. Infections of this type are, with very few exceptions, caused by the pneumococcus; and it is with such primary pneumococcal pneumonias that we are here concerned.

The Experimental Disease.—We have referred, in some detail, to the experimental study of pneumococcal infections in laboratory animals, when discussing the general problems of infection and resistance; and we may at this point confine our attention to certain experimental evidence which directly concerns the mechanism of pneumonic infection in man.

Blake and Cecil (1920*a*, *b*, *c*, Cecil and Blake 1920*a*, *b*, *c*, *d*) found that lobar pneumonia could consistently be produced in monkeys by inoculating minute amounts of pneumococcal cultures directly into the trachea. The disease so produced resembled lobar pneumonia in man: in its clinical manifestations; in its tendency to terminate by crisis; in the presence of a bacteræmic phase, which was transitory in cases that recovered, but progressive in cases that succumbed; and in the lesions found at necropsy in fatal

cases. Inoculation, by swabbing, of the nose and throat, never resulted in pneumonic infection. Monkeys which were inoculated intravenously, or subcutaneously, either died of pneumococcal septicæmia, without pulmonary lesions, or recovered without any involvement of the lungs. Blake and Cecil conclude that the normal route of infection in lobar pneumonia is *via* the bronchi, the disease spreading centrifugally through the interstitial tissues from the hilar region, and invading the alveoli from without. A similar mode of spread was postulated in rabbits by Permer (1923) and in mice by Branch and Stillman (1924). As the result of a study of human infections, an alternative view was put forward by Loeschcke (1931); the original focus of infection appeared to be near the periphery of the lung, and the infecting pneumococci seemed to be carried in œdema fluid from one alveolus to another, passing directly through the pores of Kohn. Subsequent experimental work on a number of animals has confirmed Loeschcke's view.

In dogs, pneumonia may be initiated by introducing pneumococci, together with viscous material like starch or mucin, into a terminal bronchus. The œdema fluid from the lesions, laden with pneumococci, passes directly through the alveoli and precedes the advancing consolidation. The greater the œdema in the initial lesion, the greater is the likelihood of spread; and not only are the site and spread of the lesions determined to some extent by gravity—the infected fluid tending to flow downwards—but they can be altered by appropriate changes in the posture of the sick dog (Terrell, Robertson and Coggeshall 1933, Robertson 1938, Hamburger and Robertson 1940, Robertson and Hamburger 1940). Loosli (1942) obtained similar results in monkeys, but concluded that in these animals the infection also spread from bronchiole to bronchiole as the result of repeated aspiration during breathing. In rats also the lesion advances by direct spread from alveolus to alveolus (Gunn and Nungester 1936, Wood 1941). The infected œdema fluid appears to outstrip the cellular response. At the edge of the pneumonic lesion one finds fluid and pneumococci only; then comes a zone containing active polymorphonuclear phagocytes as well; and not till the central region of consolidation is reached are active macrophages demonstrable. Since this central region may be bacteriologically sterile, it would appear that the macrophages not only ingest but kill the pneumococci.

It should be noted that the action of the starch or mucin in these experiments is not to block the bronchi and thereby induce collapse and subsequent infection in the tributary alveoli, but to protect the cocci against the defence mechanisms of the body (see Chapter 54). According to Hamburger and Robertson (1940) mere blocking of a bronchus does not predispose the corresponding alveolar portion of the lung to infection. It is probable that natural infections are initiated by the aspiration of pneumococci and mucinous material from the upper respiratory tract. Nungester and his colleagues, for example, have shown in rats that the *nasal* instillation of pneumococci and mucin does not result in pneumonia, but if by chilling or alcohol intoxication the normal mechanisms preventing the inhalation of fluids into the lung is deranged, pneumonia follows (Nungester and Klepser 1938). When mucin and pneumococci were introduced into the nose or the trachea, artificially imposed forceful inspiration induced pneumonia in about 50 per cent. of the rats tested. Forceful expiration, on the other hand, which in some respects is analogous to natural expulsive coughing, had a smaller predisposing action, for when it was imposed on the breathing of mice inoculated intratracheally, the incidence of pneumonia was only 18 per cent. (Nungester, Klepser and Kempf 1942).

As noted elsewhere, Gaskell (1927) carried out a large series of intratracheal inoculations in rabbits, and brought evidence to show that the type of infection produced in these animals is mainly determined by the virulence of the strain of pneumococcus injected, ranging, as virulence decreases, from a rapidly fatal septicæmia without pulmonary changes, through lobar and lobular pneumonia, and through lesions confined almost entirely to the bronchi and bronchioles, to a complete absence of reaction. The frequency with which pneumonia follows intranasal inoculation in mice has been found to vary both with the virulence of the strain and with the susceptibility of the animal used (see Rake 1936).

Though consideration of the epidemiology of pneumonia in man points overwhelmingly to the respiratory route of entry, the possibility of a blood-borne infection of the lung must be borne in mind. In this connection we may note that Rake (1936) by carefully balancing the virulence of the pneumococci against the resistance of the test mice induced pneumonia by intravenous inoculation; while Kempf and Nungester (1940) showed that pneumonia followed intravenous inoculation in rats previously given mucin intrabronchially.

If we collate the available experimental evidence with the numerous observations which have been carried out on the natural disease as it occurs in man (Dochez 1912, Sia, Robertson and Woo 1928, Finland and Sutcliff 1931*a*), we can construct a picture of the sequence of events in lobar pneumonia, which, however incomplete it may be, will aid us in the discussion of the results obtained in serum treatment, and in prophylactic vaccination.

It would appear that the initial phase of the infection is marked by a progressive invasion of the lung tissue, frequently associated with a detectable bacteræmia. The inflammatory reaction in the lung progresses through its successive stages; and the bacteræmia may, or may not, increase in severity. At some time between the 4th and 7th days, in favourable cases, specific antibodies begin to appear in the blood; and, coincidently with their appearance, the temperature falls by crisis, the pneumococci disappear from the blood if they were ever present in detectable numbers, and the clinical condition of the patient undergoes a striking amelioration, though abnormal signs in the lungs may persist for several days, or even weeks.

There are good grounds for believing that the degree and persistence of bacteræmia, rather than the local condition in the lungs, is the main factor in determining death or recovery. The most virulent of Gaskell's strains produced in rabbits rapid septicæmic death without pneumonic consolidation. In acutely fatal cases of pneumonia in man the lung changes at autopsy may be relatively slight; but blood cultures taken during life are, in such cases, almost always positive. The prognostic significance of a positive blood culture is one of the best-attested facts which have emerged from the bacteriological study of this disease.

Thus in a series of 448 cases, studied at the Hospital of the Rockefeller Institute, 136 gave positive blood cultures, and of these 76 died, a mortality of 55·8 per cent.; 312 gave negative blood cultures, and of these 26 died, a mortality of 8·3 per cent. (Avery *et al.* 1917). Tilghman and Finland (1937) examined a series of 1,586 cases due to all types of pneumococcus, and found that the fatality rate in bacteræmic patients was four times that in non-bacteræmic patients (see also Anderson *et al.* 1943).

In such series as these the figures given for positive or negative blood cultures must not be taken as an accurate index of the presence or absence of bacteræmia, but rather of its severity. The precise amount of blood withdrawn and, to a less extent, the method employed in cultivation will make a significant difference in the proportion of negative and positive results; and different observers have, in fact, recorded very different figures. But any one technique, employed over a considerable series of cases, will place in the positive category those patients who are suffering from a bacteræmia in excess of some limiting range, and in the negative category those whose blood is infected, less heavily, or not at all.

The Frequency of the Different Serological Types of Pneumococci in Health and Disease.—The antigenic structure of the pneumococci has been described in Chapter 24, where it is noted that the studies of Cooper and her colleagues (Cooper, Edwards and Rosenstein 1929, Cooper *et al.* 1932, Cooper and Walter 1935) resulted in the sub-division of the old "Group IV," composed of undifferentiated strains

into 29 new types; so that the differentiated and labelled types of pneumococci now run from Type I to Type XXXII, the classical Types I, II and III retaining their priority. The later work on the serological definition of strains not included in Cooper's types does not concern us here, for the present epidemiological and immunological data at our disposal relate almost entirely to Types I to XXXII. We may note that there is a close antigenic relationship between Cooper's Types VI and XXVI and between Types XV and XXX (Walter *et al.* 1940), and, as in Table 159, it is sufficient for our purposes to include Type XXVI in Type VI, and Type XXX in Type XV.

The earlier workers, distinguishing only Types I, II and III, and "Group IV" pneumococci, noted a number of characteristics in the distribution of pneumococci. In the first place, certain types predominated in infected persons, others in the nose and throat of healthy carriers. Avery, Chickering, Cole and Dochez (1917) found that in New York, during the few years prior to 1917, 80 per cent. of all cases of lobar pneumonia in adults were due to infection by Types I, II and III.

TABLE 158

FREQUENCY OF THE DIFFERENT SEROLOGICAL TYPES OF PNEUMOCOCCI IN LOBAR PNEUMONIA, AS RECORDED BY VARIOUS OBSERVERS.

Observers.	Country or District.	Period.	No. of Cases examined.	Frequency percentage.			
				Type I.	Type II.	Type III.	Group IV.
Schorer, Clark and others	U.S.A.	1917–18	101	30·7	14·9	17·8	36·6
Thomas	U.S.A.	1917–21	239	25·1	10·9	14·6	49·4
Cecil, Baldwin and Larsen	U.S.A.	1921–26	1,913	33·6	19·1	13·3	33·3
Griffith	England	1920–22	150	30·6	32·7	6·7	30·0
Glynn, Digby and Jones .	England (Liverpool)	1919–21	96	45·8	24·0	2·1	28·1
Ferguson and Lovell . .	England (Manchester)	1925–27	116	43·1	4·3	0·0	52·6

The percentage frequencies were Type I, 33·3; Type II, 33·5; and Type III, 13·0. In normal mouths, on the other hand, the percentage frequencies were Type I, 0·8; Type II, 18·2; Type III, 28·1. Group IV strains, which were associated with only 20·3 per cent. of pneumonias, were found in 52·9 per cent. of normal throats. Secondly, the incidence of the types varied from place to place, and from year to year in any one place. In Table 158 we summarize figures collected by Glynn, Digby and Jones (1923) from the literature with the addition of some collected at a later date. Cecil, Baldwin and Larsen (1927), during a 5-years' study of the distribution of the three types, noted that the frequency of Type I ranged from 21·0 per cent. to 45·2 per cent., that of Type II from 15·9 per cent. to 20·6 per cent., that of Type III from 7·9 per cent. to 17·2 per cent. and that of "Group IV" from 23·5 per cent. to 41·0 per cent.

In his study of lobar pneumonia among the mine-workers on the Rand, Lister (1917) carried out a serological analysis of 148 strains, 143 of which he allocated to 11 groups, labelled by letters, leaving 5 unclassified. The three major groups, A, B, and C, accounted for 31·0 per cent., 16·2 per cent., and 21·6 per cent. of the strains respectively, leaving

31·2 per cent. in the minor groups. Subsequent investigations have shown that Lister's Group C is identical with the American Type I, and his Group B with the American Type II; but his Group A, which was the type most frequently isolated in South Africa prior to 1916, together with a number of his infrequently occurring types, corresponded to the American "Group IV." Identities were subsequently established between most of the South African groups and Cooper's types, and Group A, for example, proved to be identical with Type V (see Ordman 1938).

In the third place, a marked difference was noted between the case-fatality rates of primary pneumococcal pneumonia according to the various infecting types. Type III infections showed a high fatality rate in the region of 40 per cent.; and the rates were progressively less with Type II, Type I and Group IV infections, in that order (see Glynn, Digby and Jones 1923, Cecil 1928, Rosenblüth 1928, Ferguson and Lovell 1928).

Since the differentiation of most of the Group IV strains into Cooper's types, many detailed surveys of pneumococci in health and disease have been made which have to a large extent confirmed the observations made on Types I, II and III alone, and which have revealed a number of additional features in the epidemiology of pneumococcal infection. We can do no more than indicate some of the outstanding results of these surveys; for details, the reviews of Finland (1942*a*, *b*) should be consulted.

All the newer types, IV–XXXII, have been found, with varying frequency, in association with respiratory infections. Types IV, V, VII and VIII are, after Types I, II, III, the most frequent types in acute lobar pneumonia of adults. In pneumonia of children and infants, the frequency of the types is different. Type XIV predominates; and Types I, VI and XIX, V, IV, III and VII are, in that order, next in frequency (see also Viktoroff *et al.* 1937).

We have recorded in Table 159 representative figures from Finland (1942*b*), showing the frequency of the Cooper types in pneumonia of adults and of children gathered from several American sources, and for healthy carriers surveyed in Germany by Gundel (1933) and in England by Straker and others (1939). The case-fatality rates are also listed for pneumonia in adults, and it will be seen that relatively high rates are recorded for infections with some of the newer types. As Finland points out, these rates do not fairly represent the capacity of the various types to produce a primary pneumonia in persons of average resistance, since many of the cases are of atypical, post-operative or otherwise traumatic pneumonia, in which the resistance of the patient has probably been substantially lowered by other causes.

With infections by all types, it has been found that the case-mortality rates are higher for atypical and secondary pneumonias than for acute lobar pneumonia; and since about three-quarters of the former are due to infection with the more recently described types (Stoneburner and Finland 1941) the high mortality rates may be due to host factors independent of the virulence of the types. If we take the occurrence of bacteræmia as an index of virulence, it is clear that of the new types, only Types IV, V, VIII, XIV and XXV approach Types I, II and III in this respect. Table 159 also shows that the type distribution in the pneumonias of children is conspicuously different from that in the pneumonias of adults. The distribution also varies within this group, according to the age of the child. Thus Nemir, Andrews and Vinograd (1936) found Type I in 7·7 per cent. and Type XIV in 36·0 per cent. of pneumonias in children under 2 years, whereas the corresponding figures in the 2–13-year-age group were 23·9 per cent. and 9·7 per cent. respectively. In healthy carriers the frequency is again different, Types III, VI, XVIII and types other than I–XXXII being commonest. The type-distribution in broncho-pneumonic infections at all ages, it should be noted, is similar to that in healthy carriers (see Finland 1937).

TABLE 159

INCIDENCE OF PNEUMOCOCCAL TYPES IN HEALTH AND DISEASE (after Finland).

Pneumococcus Type.	Pneumonia of Adults.						Pneumonia of Infants and Children		Healthy Carriers.	
	All Cases.				Bacteræmic Cases					
	Number of Cases.	Percentage of Total.	Died.	Percentage Mortality.	Number	Percentage of Type.	Number.	Percentage of Total.	Number	Percentage of Total.
I	2,573	21·4	451	17·5	655	25·4	384	15·3	42	1·2
II	888	7·4	276	31·1	344	38·7	59	2·4	36	1·0
III	1,480	12·3	536	36·2	356	24·0	103	4·1	529	14·9
IV	643	5·3	136	21·2	123	19·1	122	4·9	233	6·6
V	809	6·7	148	18·3	198	24·5	134	5·3	12	0·3
VI and XXVI	330	2·7	67	20·3	25	7·1	309	12·3	368	10·4
VII	1,116	9·3	189	16·9	194	17·4	99	4·0	71	2·0
VIII	990	8·2	164	16·6	205	20·7	40	1·6	134	3·8
IX	266	2·2	77	28·9	35	13·2	43	1·7	80	2·3
X	131	1·1	41	31·3	17	13·0	21	0·8	168	4·7
XI	129	1·1	31	24·0	6	4·4	46	1·8	133	3·8
XII	168	1·4	38	22·6	36	21·4	11	0·4	38	1·1
XIII	138	1·1	39	28·3	10	7·2	23	0·9	20	0·6
XIV	512	4·2	128	25·0	110	21·5	434	17·3	11	0·3
XV and XXX	106	0·9	28	26·4	6	5·7	64	2·6	62	1·8
XVI	91	0·8	16	17·6	5	5·5	36	1·4	5	0·1
XVII	147	1·2	47	32·0	7	4·8	33	1·3	54	1·5
XVIII	317	2·6	70	22·1	46	14·5	70	2·8	280	7·9
XIX	298	2·5	70	23·5	41	13·8	187	7·5	99	2·8
XX	192	1·6	56	29·2	24	12·5	33	1·3	64	1·8
XXI	72	0·6	12	16·7	4	5·6	42	1·7	66	1·9
XXII	105	0·9	23	21·9	9	8·6	38	1·5	144	4·1
XXIII	90	0·7	24	26·7	12	13·3	66	2·6	46	1·3
XXIV	62	0·5	12	19·4	6	9·7	18	0·7	18	0·5
XXV	114	0·9	21	18·3	26	22·8	8	0·3	25	0·7
XXVII	26	0·2	3	11·5	2	7·7	1	0·1	17	0·5
XXVIII	46	0·4	14	30·4	2	4·3	18	0·7	60	1·7
XXIX	107	0·9	25	23·4	6	5·6	31	1·2	105	2·9
XXXI	32	0·3	9	28·1	2	6·3	17	0·7	22	0·6
XXXII	22	0·2	0	....	1	4·5	4	0·2	8	0·2
Not I–XXXII	49	0·4	11	22·4	3	6·1	12	0·5	593	16·7
	12,049	100·0	2,762	22·9	2,516	20·9	2,506	100·0	3,543	100·0

We must emphasize that these figures represent only limited surveys, and that frequencies vary with time, season, locality (see, for example, Rumreich *et al.* 1943), with prevailing epidemics, and the state of immunity of the population concerned, as well as with the methods used for the isolation and typing of the pneumococci. Nevertheless, within the limits of these variations, the type-frequencies reported from various parts of the world (see Mulder 1938, Ordman 1938, Bjørnsson 1940, Lal and Chitkara 1941, Stobo and Little 1941, Siegenbeek van Heukelom and Beeuwkes 1942) bear out the general conclusions that can be drawn from Table 159.

We can divide pneumococci into the relatively virulent or " infective " types, and the apparently less virulent, characteristically " carrier " types. The infective

types are commonly associated with acute pneumococcal pneumonia, and the carrier types are commonly found in the healthy human throat. This arbitrary division is not clear cut; as Table 159 shows, "carrier" types may cause severe pneumonia, and "infective" types are carried by healthy persons.

Carrier types are established in the throat in early life (see Sutliff and Davies 1937) and are found there at all ages. Carriage of any one type may persist for years (Straker *et al.* 1939), though the type may be detected only intermittently. Several types may be carried by one person; up to seven have been recorded. In a healthy community pneumococci may be found in 40 to 50 per cent. of persons sampled once; and in nearly all persons if the sampling is repeated at intervals over a long period (see Straker *et al.* 1939, Smillie, Calderone and Onslow 1943).

The similarity of the type-frequencies in broncho-pneumonia, and in post-operative and other kinds of traumatic pneumonia, to those in healthy carriers, suggests that these infections have their source in the upper respiratory tract of the patient himself; they may, in fact, be examples of autogenous infection. The virulence of the carrier type in these circumstances is usually attributed to a non-specific reduction in the resistance of the person attacked, though the factors predisposing to infection, such as the trauma due to an anæsthetic, are not in all cases apparent. In some cases, the age factor is important; Type VI, for example, is largely a carrier type in adults, and an infective type in infants and children.

As regards the infective types, though it is possible that a persistent carrier may succumb to infection with his own type, there are many examples of epidemic outbreaks in which the responsible pneumococcus apparently infects from without; that is, in which acute lobar pneumonia spreads by contagion. When we study the carrier rates for the infective types we find, in accord with the notion of their relatively high virulence, that high rates are associated with outbreaks of pneumonia. Thus in institutional outbreaks of Type I pneumonia, Type I carrier rates of 10 and 30 per cent. have been recorded (Stillman 1916, Strøm 1932). Dauer and others (1941) observed that in a group of 50 boys, five of whom had a Type II pneumonia, the healthy carrier rate for Type II was 44 per cent. It is clear that "infective" types can spread through a community. Indeed Andrews (1937), in a survey of pneumonia of infants due to a wide variety of types, found that the types associated with the highest incidence of pneumonia in infected persons, and with the highest case fatality, were also most frequently isolated from the throats of family contacts.

The relation of the carrier state and prevalence of pneumonia is well illustrated in Smillie and Jewett's (1940) survey of familial outbreaks of pneumonia. They found that, whereas the carrier rate for carrier types like XIX or XXVII was similar for contacts and non-contacts of Type I and Type II pneumonias, the Type I carrier rate in Type I contacts was 10 per cent., but only 0·8 per cent. in non-contacts; in Type II outbreaks the corresponding Type II carrier rates were 7·4 per cent. and 0·3 per cent. (see also Rogers *et al.* 1941, 1943).

It is noteworthy that high carrier rates are often found simultaneously with outbreaks of pneumonia, and that, as in some recent surveys, a high carrier rate for a given type may even precede an outbreak (Stebbins *et al.* 1940; see also Smillie and Jewett 1942). The evidence from a number of outbreaks, in fact, suggests that acute lobar pneumonia is but one manifestation of the spread of a virulent pneumococcus, presumably from a healthy carrier of the organism; other observed manifestations are the healthy carrier state, infection of the upper respiratory tract, conjunctivitis, otitis media, and mild atypical pneumonia (see Gilman and Anderson 1938, Plummer and Ensworth 1941). The concentration of carriers, as in infections by other bacteria, to some extent determines the appearance of manifest disease. Thus, in the school outbreak recorded by Dauer and others (1941) pneumonia appeared in the group of boys with a 44 per cent. carrier rate, but not in groups with lesser rates (see also Mackenzie *et al.* 1940). This relation, however,

is by no means found consistently. Stebbins and his colleagues (1940), for example, record an epidemic prevalence of a Type V pneumonia in the absence of a continued high carrier rate, and in another population a high carrier rate of Type VI without the appearance of Type VI pneumonia.

The available evidence, then, indicates that acute lobar pneumonia is a contagious disease, which is spread by carriers and by persons with a variety of pneumococcal infections of the respiratory tract, including acute lobar pneumonia itself. We have little precise knowledge of the circumstances that determine infection of the lung in a given person. Certain types of pneumococcus appear to infect more readily than others. As regards the part played by immunity of the host, we have noted some factors, like age, which are associated with resistance to various types of pneumococci. The rôle of specific immunity we shall consider in later sections of this chapter.

The Treatment of Pneumonia with Antipneumococcal Serum.

The sera employed in the treatment of pneumonia are prepared by injecting horses with whole cultures of pneumococci of one or more of the different serological types. All the laboratory evidence suggests that anti-pneumococcal immunity is type-specific; and we should not therefore expect a serum prepared against any one type to exert a curative effect on an infection caused by any other. Many of the early trials in man were, in fact, carried out with a " polyvalent " serum prepared by immunizing horses against Types I, II and III. It will be convenient to consider the results obtained in trials with this, and other, sera before discussing the question of how such antisera should be standardized and utilized.

We have quoted numerous experiments that establish the protective action of a type-specific antiserum as tested in the rabbit or mouse, and a few that indicate a curative action when the serum is injected shortly after the establishment of an experimental bacteræmia. In either case it has been shown that the serum increases the efficacy of the normal clearing mechanism and suppresses, or cuts short, the bacteræmic phase.

Of particular interest, from our present point of view, are the following observations. Cecil and Blake (1920*e*) studied the effect of serum treatment on experimental lobar pneumonia in monkeys, induced by the intratracheal inoculation of virulent Type I pneumococci. They found that the intravenous injection of the specific antiserum exerted a prompt therapeutic effect, freeing the circulating blood from pneumococci, shortening the infection and greatly reducing its severity. Of 5 treated monkeys all recovered while the controls died. Curphey and Baruch (1932) infected rabbits by the intradermal injection of virulent Type I pneumococci, and studied the effect of the subsequent injection of a Type I antipneumococcal serum by the intravenous or intramuscular route. With the intravenous injection of 400 units 24 hours after infection, 1 of 13 animals survived; with 800 units 12 of 32 animals survived; with 2,500 units 14 of 19 animals survived. With shorter intervals between infection and the administration of serum smaller doses sufficed to ensure the survival of a considerable proportion of the animals.

This method of treatment has since 1915 received an extensive clinical trial in man, particularly in America. It was, however, some years before its value was established with an approach to certainty, and a serious attempt was made to assess its effect in terms of a percentage reduction in mortality. Even now we can offer only approximate estimates, and some few observations are hard to fit into the general picture. The history of the serum treatment of pneumonia

affords an illuminating example of the neglect of a useful therapeutic measure that may follow a failure to plan early trials in such a way as to allow significant but undramatic effects to be measured with some approach to accuracy.

The earliest considerable series of cases are those recorded by Avery and his colleagues (Avery *et al.* 1917). In 107 cases of serum-treated pneumonia there were 8 deaths, a mortality of 7·5 per cent. There were no controls; but a favourable conclusion with regard to the influence of serum on mortality was based on the contrast between the observed case-fatality rate and the rates of 25–30 per cent. recorded by these workers before they commenced the use of serum, and by other observers elsewhere. This method of comparison is, however, not valid. The variability of the case-mortality rates in different outbreaks of pneumonia is well known; and the records of serum-treated cases show a similar variability, though, for the most part, over a smaller range. Thus Wadsworth (1924), commenting on a series of collected figures, notes case-fatality rates in serum-treated patients varying from 6·0 to 18·0 per cent. Clearly the only satisfactory way of assessing the effect of serum is to compare treated with untreated cases in the same place and at the same time. A considerable series of trials have been carried out along these lines. Concentrated polyvalent antipneumococcal sera containing antibodies for Types I, II and III have been used, the Type III antibody being present in relatively low concentration.

In a series recorded by Cecil (1928) the data were collected as follows. During the winters of 1920, 1921 and 1922 all cases of pneumonia admitted to six medical wards were treated with Huntoon's polyvalent antibody solution, while all cases of pneumonia admitted to six other wards were treated on ordinary medical lines without serum therapy. The results are recorded in Table 160.

TABLE 160

COMPARISON OF CASE-FATALITY RATES IN TREATED AND UNTREATED CASES OF LOBAR PNEUMONIA (CECIL).

Type of Pneumococcus.	Antibody Wards.			Control Wards.			Difference.	Diff./S.E. Diff.
	Cases.	Deaths.	Deaths Percentage.	Cases.	Deaths.	Deaths Percentage.		
I	158	21	13·3	162	36	22·2	8·9 ± 4·27	2·09
II	83	23	27·7	67	27	40·3	12·6 ± 7·74	1·63
III	73	29	39·7	60	24	40·0	0·3 ± 8·5	—
Group IV . .	110	18	16·4	121	29	24·0	7·6 ± 5·2	1·46

There is a clear suggestion that Type I and Type II pneumonias were benefited by the serum, while Type III pneumonias were not. Only in the case of Type I pneumonia does the difference between the treated and untreated groups as compared with its standard error attain a value that would be conventionally regarded as statistically significant. The suggestion that some therapeutic effect was exerted on pneumonias due to infection with strains belonging to the antigenically heterogeneous Group IV is not in accord with our expectations; but the difference in this case falls short of statistical significance.

A very valuable record is that provided by Park, Bullowa and Rosenblüth (1928). The serum employed was Felton's concentrated polyvalent antipneumococcal serum, and random sampling was ensured, as far as possible, by giving serum to alternate cases in order of admission. The relevant figures are set out in Table 161.

TABLE 161

Type.	Hospital.	Treated.		Untreated.		Difference Percentage.	Diff./S.E. Diff.
		Cases.	Deaths Percentage.	Cases.	Deaths Percentage.		
I	[1] Combined	266	19	249	33	14 ± 3·8	3·7
	Bellevue	144	19	132	33	14 ± 5·3	2·6
	Harlem	114	20	109	34	14 ± 5·8	2·4
II . . .	Combined	176	35	165	45	10 ± 5·3	1·9
	Bellevue	107	39	95	53	14 ± 6·9	2·0
	Harlem	61	30	63	32	2 ± 8·3	0·2
III . . .	Combined	82	33	92	29	− 4 ± 7·0	0·6
	Bellevue	32	38	52	27	− 11 ± 10·6	1·0
	Harlem	47	32	38	32	0 ± 10·2	—
Group IV .	Combined	313	24	324	26	2 ± 3·4	0·6
	Bellevue	131	30	128	40	10 ± 5·2	1·9
	Harlem	171	20	190	15	− 5 ± 4·0	1·2

[1] The combined figures include a small series of cases treated at the New York Hospital.

Taking the combined experience it will be noted that the concentrated serum exerted a significantly beneficial effect on Type I pneumonia. In Type II pneumonia the effect is more doubtful. The combined experience clearly suggests a beneficial effect; but this is due almost entirely to the results obtained in Bellevue Hospital. In Harlem Hospital over the same period of time serum-treated Type II pneumonias showed no advantage, a fact that emphasizes the danger of putting trust in small numbers or in single trials. The Type III cases received no benefit from the serum. The Group IV cases were unaffected as judged by the combined experience. In Bellevue they seemed to receive some benefit. In Harlem they fared worse than the controls. We need not then attach much importance to the apparent therapeutic effect in Group IV cases noted by Cecil.

Cecil and Plummer (1930) record a series of Type I pneumonias treated with Felton's serum between 1924 and 1929. Of 239 treated cases 20·1 per cent. died; of 234 untreated cases 31·2 per cent. died, giving a difference of 11·1 ± 4·01 per cent. With Type II pneumonia the results were curious (Cecil and Plummer 1932); of 252 treated patients 40·5 per cent. died; and of 253 untreated patients 45·8 per cent. died. But analysing the results season by season, the differences in case mortality were 15·0 per cent., 7·3 per cent., and 15·1 per cent. in favour of the treated, and then 7·5 per cent. and 4·8 per cent. in favour of the untreated cases. In a subsequent season 1930–31 the trial was continued on the alternate case method, limiting the treated and control groups to cases of Type II pneumonia admitted within 72 hours of onset. Of 21 treated cases 3 died; of 20 control cases 13 died (see also Cecil 1937).

The therapeutic value of Types I and II antisera depends to some extent on the age of the patient treated. It has been found, for example, that whereas the case mortality in the 20–40 age group is significantly lower in treated than in untreated cases, there is little difference in the 40–60 age group (Report 1934; see also Langley *et al.* 1937).

Horse antisera against Types IV, V, VII, VIII and XIV pneumococci have also been tested in the United States in controlled series of cases: the results indicate that serum treatment reduces the mortality due to Type V and VII pneumonia by about half (see Bullowa 1937, Finland and Brown 1939*a*, Benjamin *et al.* 1937, Kohl and Reitzel 1936). The antisera to Types IV, VIII and XIV were also reported to have a favourable effect, though the evidence was for various reasons less clear cut.

More recently, therapeutic rabbit antisera, as developed by Horsfall and his colleagues (1937), have largely replaced horse antisera in the United States. Antisera can be prepared more quickly in the rabbit, in some cases (e.g. Types II and VIII) with higher titres of protective antibodies than are usually obtained in the horse; and, after a refining process which removes substances that give rise to thermal reactions, they can be used safely in man. The rabbit antibody globulin particle is less than one-quarter the size of the horse antipneumococcal antibody, and thus possesses the theoretical advantage of being more likely to diffuse readily through the infected tissues of the patient (see Chapters 7 and 47). Rabbit antisera to Types I, II, V and VII have proved to be at least as effective as the corresponding horse antisera; and there are indications that antisera to Types III, IV, VIII and XIV have a beneficial effect. Antisera to all the remaining Cooper types have been tested, but in most cases the results are inconclusive, partly owing to the small number of patients so far treated, and partly to the complicating factor of sulphonamide treatment. Comparisons of case-mortality rates, however, suggest that specific serum is most effective in infections with Types XII, XX, XXV and XXVI; less so with Types IX, X, XVII, XVIII, XXIV; and fairly effective with Types VI, XIX, XXII, XXIII, XXIX and XXXII (Finland 1942*b*; see also Madsen and Vammen 1940).

The results of clinical trials are in entire accord with the view, based on laboratory experience, that the therapeutic effect of each of these sera is type-specific, depending in the main on the content of the antibody acting on the capsular, type-specific, polysaccharide antigen.

If, then, we intend to use an antipneumococcal serum we must determine the type of infecting pneumococcus at the earliest possible moment.

The method of typing usually employed is to inject a specimen of sputum into the peritoneum of a mouse, to kill the animal after 6 to 8 hours, and to carry out agglutination and precipitation tests with the sediment and supernatant fluid of the peritoneal washings. This method can be made more rapid by using slide agglutination followed by staining (Sabin 1929), and noting the swelling of the capsule that results from the action of the antiserum, as well as the agglutination itself. This capsular-swelling reaction, which was first described by Neufeld (1902), can be applied to specimens of sputum direct (see Armstrong 1932, Logan and Smeall 1932, Sabin 1933 Beckler and MacLeod 1934, Cooper and Walter 1935, Bullowa 1935, Mulder 1935, Vinograd, Nemir and Park 1936, Cruickshank and Montgomery 1938). In experienced hands, the agreement of the results of typing by the direct swelling reaction and the indirect after mouse-inoculation varies between 75 and 90 per cent.

Type-specific polysaccharides, detected by precipitin tests with specific antisera, are found in the urine of patients with pneumonia (Dochez and Avery 1917). In the early stages of the disease, positive reactions are too infrequent to be depended upon as an indication of the infecting pneumococcal type (Cruickshank 1938, Cruickshank and Montgomery 1938).

As regards the dose of serum and the number and frequency of the injections that should be administered, there are good reasons for believing that these factors are of primary importance. We might suppose from our knowledge of passive protection in the rabbit or mouse that a comparatively small dose of serum would suffice to render the pneumococci sensitive to phagocytosis. But the experiments of Curphey and Baruch (1932) show that, with antibacterial as with antitoxic sera, the effective curative dose is a high multiple of the protective dose. It is probable that this is in part due to the fact that the specific antigen, or hapten, accumulates in the lesions and in the blood in considerable quantities during a pneumococcal infection and exerts an aggressive effect, combining with the corresponding antibody and so diverting it from the living pneumococci.

Thus, Park and Cooper (1928) note that, when an antipneumococcal serum is injected into a normal person, the specific antibody disappears relatively slowly from the circulating blood; so that one-quarter to one-half of the amount administered is still present 24 hours after an intravenous injection. In severe cases of pneumonia the course of events is quite different; the specific antibody is rapidly neutralized. If a polyvalent serum is administered it will be found that the antibodies corresponding to types other than that responsible for the infection can be detected in the circulating blood for relatively long periods, as in normal persons (see also Langley *et al.* 1936).

By suitable titration in cases of Type II pneumonia, Park and Cooper were able to show that the circulating antigen alone would require several thousand units of antibody for its neutralization. In Chapter 48, we noted the enormous amounts of specific polysaccharides estimated to be present in the pneumonic lungs, particularly in Type III infections, amounts that for their full neutralization would require litres of concentrated antibody; and though it is unlikely that all the antigen held in the tissues must be neutralized before any injected antibody is available for direct action on the invading pneumococci, the aggressive action of a substantial part of it will necessarily affect the outcome of serum therapy.

In this connection, it should be noted that Cruickshank and Montgomery (1938) demonstrated that the presence of urinary, and, therefore, presumably of circulating, polysaccharide in the early stages of pneumonia was of bad prognostic significance. The death rate in 62 such patients was 38·7 per cent., whereas, of 53 patients whose urine was persistently free, only 1 died.

Park and Cooper recommend that every pneumonia patient should, on admission to hospital, receive 10,000 units of Type I and Type II antibody administered intravenously. So long as the temperature remains high, the injections should be continued every 8 to 12 hours. When the type of the infecting pneumococcus has been determined a monovalent serum may be substituted for the polyvalent preparation. If the infection is found to be due to a type of pneumococcus against which no specific serum is available, the injections should be discontinued (see also Bullowa 1937).

The Standardization of Antipneumococcal Sera.

In the original attempts to measure the potency of such a serum (see Neufeld and Händel 1909, Neufeld and Schnitzer 1927–28) titrations were made in mice both with a constant dose of serum and a varying dose of pneumococci, and with a constant dose of pneumococci and a varying dose of serum.

These and later experiments (see Felton 1930*a*) have made it clear that the relation between the number of pneumococci injected and the dose of serum necessary to protect against them is not of a simple linear kind, *i.e.*, if x ml. of serum will protect against y pneumococci it does not follow that nx ml. of serum will protect against ny pneumococci. Felton's experiments suggested that, under optimal conditions, this linear relationship held over a limited range; but the results obtained by Smith (1932) indicate that it is very unsafe to assume that if one serum in a particular dose gives a certain degree of protection against n pneumococci, and another serum in the same dose gives the same degree of protection against $2n$ pneumococci, the second serum is twice as potent as the first. He found, for instance, that with a constant dose of a particular serum and varying doses of pneumococci, the death rate in a group of 50 mice receiving 12,500 cocci was greater than that in another group of 50 mice receiving 125,000 pneumococci, while in another experiment a hundredfold increase in the number of pneumococci, keeping the dose of serum constant, failed to cause any significant increase in the percentage mortality.

It happened, however, for reasons that are not altogether clear, that the method of varying the dose of pneumococci was at first adopted in testing antipneumococcal sera

(see Prausnitz 1929). Sera were not titrated in terms of units, but a limit of potency was laid down which had to be attained before a serum was regarded as satisfactory for issue. In Germany this limit was protection by 0·2 ml. of serum against 0·1 ml. of a culture of such virulence that 0·000,001 ml. was fatal for untreated mice. In America it was protection by 0·2 ml. of serum against 0·1 ml. of a culture of such virulence that 0·000,000,1 ml. was fatal for untreated mice. A method that does not assign a value in terms of units to each batch of serum issued is clearly unsatisfactory from the clinical point of view, and of more recent years attempts have been made to improve the method of standardization, though no international agreement has yet been reached.

Felton (1925, 1928, 1930*a*, *b*, 1931) has carried out extensive studies on this problem, adopting the method of testing varying doses of the serum to be standardized against a constant dose of pneumococci and defining the unit of serum as the least amount that will protect mice against 1,000,000 lethal doses of a virulent pneumococcal culture. His studies, and those of Parish (1930), Trevan (1930), Falk *et al.* (1931) and Smith (1932), make it clear that the unit value of a serum cannot be accurately determined by direct titration against any particular pneumococcal culture. Smith, for instance, in repeated titrations of a particular serum against a particular strain of pneumococcus found that the amount of serum necessary to protect 50 per cent of mice against the test dose of culture employed varied between 0·0009 ml. and 0·0034 ml. Felton (1930*a*) would ascribe such divergent results, particularly when different strains of pneumococci are employed, to independent variation of virulence and of capacity to unite with antibody.

This difficulty may in any case be avoided by comparing an unknown serum with a standard serum, and assigning a value to the former, not in terms of the particular dose of pneumococci against which it protects, but in terms of the arbitrary unit value assigned to the standard preparation. Any variation in the culture will presumably affect the neutralizing power of the standard and the unknown serum to the same extent; and it is probably a fair assumption that, if n ml. of the standard and $2n$ ml. of the unknown serum each give a mortality of 50 per cent. when injected into an adequate sample of mice together with the same dose of the same pneumococcal culture, the unknown serum contains half as many units of protective antibody as the standard.

The titration curves recorded by Parish (1930), Trevan (1930) and Smith (1932) make it clear that this method allows an assay within a reasonable degree of accuracy provided that an adequate sample of mice is injected with each dose. Actually the standardization of an antipneumococcal serum to within a margin of error of $\pm$ 30 per cent. demands the use of 40 mice for each dose of the unknown serum tested, and another 40 mice for each dose of the standard serum with which it is to be compared.

It may be added that the common practice is to inject the serum and culture simultaneously into the peritoneum and to use 0·0025–0·1 ml. of culture as the test dose, according to its virulence and bacterial content. The virulence should be maximal, so that 10 pneumococci or less will kill untreated mice, and the inoculum should correspond to 500,000–5,000,000 lethal doses.

An important saving in mice would obviously be effected if we could substitute an *in vitro* for an *in vivo* test. The type-specificity of the protective action clearly suggests that the effective antibody is that which corresponds to the type-specific polysaccharide; but, equally clearly, we must establish this identity by direct experiment before we can accept an *in vitro* estimation of this antibody as affording an adequate measure of protective value.

Certain of the earlier observers (see for instance Wadsworth 1917) reported a complete and puzzling lack of correlation between agglutinin titre and protective power.

It seems likely that some particularly unfavourable conditions must have affected such results as these; since Falk *et al.* (1931) and Felton (1931) record a close parallelism between agglutinin titre and protective power in mice. Felton, testing 39 sera by both methods, reports a correlation coefficient of + 0·80; but his protocols show a few markedly divergent results.

Since the isolation of the type-specific polysaccharide hapten of Heidelberger and Avery (1923) attempts have naturally been made to correlate precipitin content with protective power, and these have, on the whole, given better correlation than the agglutinin estimations (see Sobotka and Friedlander 1928, Friedlander *et al.* 1928, Zozaya *et al.* 1930, Heidelberger *et al.* 1930, Falk *et al.* 1931, and Felton 1931). Few of these comparative titrations can, however, be regarded as an adequate test, since the method of optimal proportions was not followed in determining the precipitin content of the serum.

Smith (1932), using the optimal-proportion method of Dean and Webb (1926) in the precipitin estimations, and injecting adequate samples of mice (40 for each dose of serum) in the protection tests, compared several Type I antipneumococcal sera with a particular serum taken as a standard. With unconcentrated sera, the correspondence between precipitin and protective antibody content was good. With concentrated sera, however, the protective power was greater than that indicated by the precipitin content (see also Hartley *et al.* 1933, Smith *et al.* 1934, Goodner and Horsfall 1937). The use of selected antigenic fractions of the pneumococci in precipitin tests for the standardization of sera has many advantages over the laborious and expensive mouse-protection tests, and may eventually replace them. But it must be emphasized that the establishment of the identity of the antibody to the specific polysaccharide and protective antibody is but a first step in the transition to *in vitro* tests. The antigenic preparation used must be constant in composition and serological reactivity; the lability of the acetyl group in the Type I polysaccharide (see p. 280) provides an example of the difficulties to be expected. Moreover, as we saw in Chapter 7, the antibodies in native antisera are often heterogeneous. In a given antibody fraction, protective and precipitating powers may be widely discrepant; and even where we find a dependable parallelism of the two in native antisera, it may, as suggested by the results of Smith's experiments (see also Lyall 1941), be disturbed during their concentration and refinement for therapeutic purposes.

Chemotherapy of Acute Lobar Pneumonia.—Since the demonstration of its efficacy in acute lobar pneumonia by Evans and Gaisford (1938) sulphapyridine has been successfully used in the treatment of all kinds of pneumococcal infections, including acute lobar pneumonia (see, for example, Ordman 1939, Ormiston *et al.* 1942, Anderson 1943).

Sulphadiazine and sulphathiazole appear to be equally effective (see Spink 1942); and sulphamezathine (see Melton 1944) and sulphapyrazine (Ruegsegger *et al.* 1943) have been reported on very favourably. Penicillin is likewise said to be of value (Tillett *et al.* 1944). The relative efficacy, ease of administration, independence of the infecting type of pneumococcus, and cheapness of the sulphonamides, has tended to a replacement of serum therapy by chemotherapy. As we have seen in Chapter 54, there is every reason to believe that different processes are concerned in the antibacterial action of sulphonamides and of specific antibody. We might, therefore, on *a priori* grounds expect that the two together would be more effective than either alone. Finland and Brown (1939*b*), for example, demonstrated a synergic effect of the two in combination on the bactericidal power of human blood. Wright and Gunn (1940), on the other hand, in a test of experimental Type III pneumonia in rats, found sulphapyridine to be more effective than serum, and the combination of serum and drug to be no better than sulphapyridine alone. Clinically there are few reports that indicate a marked synergic effect. Finland (1942*b*) has reviewed the results of a number of clinical trials in America. In a general summary of infections by all the Cooper types, the mortality was 30·5 per cent. in 2,473 untreated

cases, 14·9 per cent. in 9,053 serum-treated cases, 7·6 per cent. in 12,547 sulphonamide-treated cases and 13·4 per cent. in 12,167 patients receiving combined therapy. For individual types, the figures were more variable, serum treatment being more successful than sulphonamides in infections with the less common Cooper types. The case mortality in the group receiving combined therapy was almost double that in the group receiving sulphonamide alone; but this does not necessarily mean that serum had a deleterious effect, for, as Finland points out, it is probable that serum treatment is combined with sulphonamides mainly in patients whose illness, by reason of its severity or other prejudicial factors, is not responding to the drug; therefore a higher mortality is in any event to be expected in this group (see also Shackman and Bullowa 1943). But aside from case-mortality rates, there is clinical evidence of an early crisis and of other improvement induced by type-specific serum, which suggest that serum therapy is a useful adjuvant to chemotherapy, especially in patients with bacteræmia.

It has been shown that drug-fast pneumococci are sometimes produced by sulphonamide therapy of experimentally infected animals (Chapter 54). The induction in man of pneumococci sufficiently drug-fast to be a danger to the patient himself, or to persons who become infected with the drug-fast strain, appears to be a rare event (Grumbach and Hegglin 1942, Hamburger *et al.* 1943) though Frisch, Price and Myers (1943) record cross-infection of a patient with a sulphadiazine-fast Type VIII pneumococcus. It is not possible to judge the magnitude of the problem until further evidence is forthcoming.

Other Possible Methods of Specific Treatment.

In view of the relatively poor therapeutic powers of Type III antipneumococcal sera, and the relative frequency of this type of infection, it is perhaps worth noting an alternative method of treating this type of infection; though, so far as we are aware, no trials in man have yet been made (see Cecil *et al.* 1936). This method depends on an observation made by Dubos and Avery (1931). These workers isolated a bacterium which, when grown in a synthetic medium containing the specific polysaccharide of Type III pneumococcus as the source of carbon, elaborates an enzyme that specifically attacks this hapten. The enzyme has no bactericidal action on Type III pneumococci, but it attacks the polysaccharide hapten as it is produced by the growing bacterial cells. When mice are injected with the specific enzyme together with a fatal dose of virulent Type III pneumococci they are effectively protected (Avery and Dubos 1931); and the protection is sharply type-specific. The enzyme also exerts a curative action in mice if injected during the earlier stages of infection. Goodner, Dubos and Avery (1932) studied the curative action of this enzyme on Type III infections in the rabbit, induced by the intradermal injection of a virulent strain. This type of infection, which starts as a spreading local lesion and leads, in untreated controls, to an acute bacteræmia fatal on about the 3rd or 4th day, offers obvious advantages for studies of this kind as compared with the acutely fatal peritoneal infection in the mouse. The enzyme, injected intravenously in adequate dosage 24 hours after the intradermal injection of pneumococci, was found to have a striking curative effect. In one series of tests, for instance, one of 19 treated animals died as compared with 36 of 38 untreated controls. Goodner and Dubos (1932) studied the relation between the degree of bacteræmia 24 hours after infection and the curative dose of enzyme given at this time. Of 10 rabbits showing 3–10 pneumococci per ml. of blood and receiving 2·5–100 " units " of enzyme all survived. Of 11 rabbits showing 103–630 cocci per ml. of blood, 5 that received 4–10 units died, 6 that received 20–100 units recovered. Of 9 rabbits showing over 13,000 cocci per ml. of blood and receiving 5–100 units of enzyme all died. Francis, Terrell, Dubos and Avery (1934) record a series of observations on the curative effect of this enzyme on experimental Type III pneumonia in the monkey. In 68 untreated monkeys the mortality was 50 per cent.; in 40 treated monkeys it was 20 per cent. (see also Dubos 1932, 1935, Dubos and Bauer 1935).

Prophylactic Immunization against Pneumonia.

The records of antipneumococcal immunization in laboratory animals are copious, and their significance is unequivocal. It is clear that susceptible animals, such as mice and rabbits, can be immunized with almost uniform success against infection by the usual routes. More immediately pertinent, perhaps, are the observations of Cecil and Blake (1920*a*, *b*, *c*) and Cecil and Steffen (1923, 1925), which showed that monkeys could be immunized with a considerable measure of success against intratracheal infection with Type I or Type II pneumococci, though the results with Type III were less promising (see also Chapter 50).

As in serum treatment, however, all the evidence suggests that the immunity acquired is predominantly type-specific ; and it is clear that, with so heterogeneous a species as the pneumococcus, this factor will seriously limit the usefulness of mass immunization. It is possible that vaccination against a few prevalent types might produce a significant decrease in the incidence of the disease, even though it failed to eliminate it, or to reduce it to negligible proportions ; but this we can only tell by experience.

The records of field trials on man tell a rather puzzling story. The frequency of lobar pneumonia, though very high in the aggregate, is not concentrated on particular age groups or confined in considerable part, as are diphtheria and scarlet fever, to schools and institutions. There are, however, certain environments that entail a particularly high risk of infection ; and, in some of them, trials have been carried out on a considerable scale.

The most extensive data are those provided by the report of Lister (1917) on the result of vaccination against pneumonia among the native labourers on the Rand mines. The numbers at risk were large ; but over the greater part of the period of trial the natives were not divided into inoculated and uninoculated groups. An earlier trial (see Orenstein 1931), in which 55,900 natives of various tribes had been divided into two equal groups, one inoculated and the other uninoculated, had shown a pneumonia incidence of 16·4 per 1,000 among the inoculated, 20·6 per 1,000 among the controls. As a result, wholesale inoculation was commenced in many mines, and the only basis of comparison available is that between the incidence of pneumonia in a particular mine before and after the institution of active immunization. As we have seen, this method of comparison is liable to many errors. There was, in fact, a definite fall in the incidence of pneumonia following these mass inoculations ; but the pneumonia death rate on the Rand had fallen sharply from the high level of 1907–11 before mass immunization was instituted, and there is no need to assume that the fall in death rate from 1916–20 was other than a continuation of the trend that was already well marked over the period 1911–14. Orenstein (1931) would attribute the whole downward trend to circumstances other than immunization, in particular to the decrease in the recruitment of tropical natives since 1911. If, as seems probable, we can attach little significance to the secular change in incidence or mortality we are left with ancillary evidence concerning the prevalence of particular infecting types in the pre- and post-inoculation periods. Lister had previously classified the strains of pneumococci isolated from the Rand miners into a series of types, of which A, B and C accounted for more than 70 per cent. of cases. Subsequent investigations have shown that Lister's Types A, B and C correspond to the classical Types V, II and I. Lister at first gave three subcutaneous injections consisting of 2×10^9 cocci of each type. In a particular trial 82 cases of pneumonia occurred among some 10,000 inoculated natives ; but none of these were due to infection with Type A, B or C. The subsequent history of events on the Rand is, however, very difficult to interpret. In 1917 a change was made in the constitution of the vaccine, each dose containing 1,000,000,000 cocci of each of eight different types

(see Ordman 1931). The pneumonia death rate continued to fall till about 1920–22, and then rose; the rise, though not great, was most marked in the last 3 years of the period under review—1927, 1928 and 1929. Orenstein (1931) notes that, in 1929, inoculation was discontinued in certain mines. The mines in which inoculation was continued had a higher attack rate in 1930 than in 1928, while the mines in which inoculation had been stopped had a lower attack rate. It may be, as Ordman (1931) has suggested, that the more recent failure in immunization was due to a change in the type of infection, not only in the prevalent types of pneumococci but in the rôle played by other bacterial species. In so far as this is the case, it illustrates the difficulty of immunizing against the clinical syndrome "pneumonia," which after all is the practical problem to be faced.

Environmental conditions particularly favourable to the occurrence of lobar pneumonia were provided in some of the camps and depôts during the war of 1914–18. Cecil (1925) reviews the results of a considerable series of mass inoculations carried out in America during the period 1918–19. As in so many of the medical records of the war, military exigencies often prevented the fulfilment of the conditions needed for a satisfactory statistical comparison. At Camp Upton, among 12,519 vaccinated men no case of pneumonia due to Type I, II or III pneumococci occurred during the trial period, while 26 cases occurred among 20,000 unvaccinated controls; but the incidence in the controls was very low, and the significance of the difference is rendered doubtful by the fact that pneumonias due to other types of pneumococci, and streptococcal pneumonia, were also more frequent among the controls than among the inoculated, a result that can hardly be attributed to the inoculation. At another depôt 38 cases of pneumonia were recorded among 48,849 vaccinated men, an incidence of 83·5 per 100,000; and 83 cases among 49,463 unvaccinated men, an incidence of 168 per 100,000. The types of infecting pneumococci in this series are not specified.

A third large-scale trial has been recorded by Malone (1925) and by King (1925). This was carried out on Indian troops in the Baluchistan district. The vaccine employed contained Type I and Type II pneumococci, 2,500,000,000 of each, and two injections were given. Among 2,996 inoculated men the pneumonia incidence was 9·346 per 1,000; among 5,227 uninoculated controls it was 10·714 per 1,000, a quite insignificant difference. The lack of protection could not be ascribed to a difference in the incidence of the different pneumococcal types among the inoculated and uninoculated; analysis of the type-frequencies showed that there was no significant evidence of protection against Types I and II.

The results of immunization with specific polysaccharide are on the whole as equivocal as those with vaccines. The studies of Felton and his colleagues in America show clearly that Type I and Type II polysaccharides may be as effective as a vaccine of whole bacilli in stimulating the production of protective antibodies in man, though individual responses are highly variable (Felton 1938, Felton, Cameron and Prather 1941, Felton *et al.* 1941). A field test, involving several thousand men in both control and inoculated groups, was made in two winter camps. In one, during an average period of observation of 10 weeks, the incidence of pneumonia in the uninoculated was 15·69 per 1,000 man-years of exposure to the prevailing infections, and 1·73 per 1,000 man-years in the inoculated; in the other, over an average period of observation of 17 weeks the corresponding rates were 7·28 in the uninoculated, 4·34 in the inoculated. The camps differed in many respects, but it is possible that the smaller effect of immunization in the second camp was the result of the longer period of observation; if so, it indicates that the protective effect of the inoculation is of relatively short duration. There was some evidence of protection against heterologous as well as homologous types (Ekwurzel *et al.* 1938). Siegel and Muckenfuss (1941), working under different epidemiological conditions, observed a community of feeble-minded persons for four years, and could find no evidence that the incidence of pneumonia, whether due to homologous or heterologous types, was affected by immunization with a mixture of Types I and II, and later Type III, polysaccharides.

It may be added that in a number of institutional epidemics due to a known type

of pneumococcus, immunization of the population at risk by type-specific vaccines or polysaccharides has been followed by marked diminution or cessation of the outbreak (Bonne 1936, Smillie 1936, Smillie, Warnock and White 1938, Dauer *et al.* 1941). However, the natural and sometimes sudden decline of an epidemic, especially in closed communities, is so common a phenomenon that the value of the vaccines in these cases cannot be assessed with any confidence.

In summary, it would seem fair to conclude that there is presumptive evidence, based on animal experiments, that immunization with particular types of pneumococci will afford some increase in resistance to infection with these types. This evidence has not been strengthened by the results of recorded trials in man; and it would seem that, with our present methods, the increase in resistance attained is either slight or transient. It may be, as Felton and Prather (1939) suggest, that the small proportion of persons who react feebly to a pneumococcal antigen are naturally more liable than the good reactors to infection with that type of pneumococcus; so that unless feeble reactors are selected for intensive immunization, the community as a whole may not benefit from immunization. Good reactors presumably respond rapidly to infection by an epidemic type of pneumococcus, and become carriers. The fact that, in a number of small outbreaks, a high proportion of the contact-carriers have been found to develop type-specific protective antibodies (see Finland, Brown and Barnes 1940, Plummer and Ensworth 1941, Rogers *et al.* 1943) is in accord with this view.

It is possible that chemoprophylaxis will prove more effective than vaccination in conferring protection on specially exposed or susceptible populations; but though a number of claims for the value of this method have been made, no one of the large-scale trials of sulphonamide on which they are based has been carried out under such conditions as to carry complete conviction.

Skin Reactions in Pneumonia.

We may close this section by noting certain observations which, though not immediately applicable to the practical control of the disease, have considerable theoretical significance.

Tillett and Francis (1929) tested the effect of intradermal injections of purified pneumococcal polysaccharide, and of pneumococcal protein, into patients suffering from pneumonia. They found that convalescent patients reacted to the injection of the polysaccharide by developing a wheal-and-erythema reaction which appeared within 30–60 minutes and then rapidly faded. This reaction was type-specific. Injection of pneumococcal protein, on the other hand, produced a delayed erythematous reaction reaching its height in 18–24 hours. This reaction was not type-specific. Finland and Sutliff (1931*a*, *b*) confirmed these observations, noting that the wheal-and-erythema reaction appears at or about the time of the crisis in patients who recover, while it is usually absent in cases that end fatally. In certain groups of subjects in whom repeated tests were carried out, they found that skin-sensitivity to the later injections appeared to develop as the result of the earlier ones. They also observed (Finland and Sutliff 1931*a*) that skin sensitivity was induced by the intravenous administration of an anti-pneumococcal serum. Francis (1933) records an extended experience of the occurrence of the immediate wheal-and-erythema reaction in 53 cases of Type I pneumonia, 48 of which were treated with Type I antipneumococcal serum. The results confirmed his previous finding that the appearance of the reaction has a favourable prognostic significance, and that its absence is suggestive of a fatal issue. The appearance of the reaction is correlated with the presence of specific antibodies in the blood, in the sense that the skin reaction is never present if antibodies are absent; but the skin test may

be negative though circulating antibodies are present, and its absence, even in such cases, seems to retain its bad prognostic significance. Finland and Dowling (1935) record a further study of the response to the intradermal injection of pneumococcal polysaccharide, in which they were able to show that the injection of purified polysaccharide, prepared by two different methods, induced characteristic skin sensitivity and, in addition, led to the appearance of specific antibodies in the serum. Subsequent work has not confirmed the high degree of correlation between the presence of antibody and the immediate skin reaction, particularly in naturally immune persons, though the correlation is better in specifically immunized persons (see Finland and Brown 1938, Felton and Prather 1939).

These observations are of interest in that they provide confirmation of the antigenicity of the polysaccharides in man, and indicate that a specific cell activity, associated with, but not entirely determined by, the presence of antibodies in the blood, is an important factor in the protective mechanisms of the tissues. Their significance is clearly not confined to the particular case of pneumonia.

Felton and Prather (1939) record that in man a *negative* reaction to an intradermal injection of a compound of specific polysaccharide with homologous antibody may be used to indicate the presence of antibody in the circulation. It remains to note that the presence in the serum of the " C-reactive " substance during the acute stage of pneumonia and other febrile diseases (see Chapter 49) is accompanied by a skin reactivity to the C-substance of the pneumococcal cell (Francis and Abernethy 1934).

Pneumonia in the Lower Animals due to *H. bronchisepticus*

In 1911 M'Gowan isolated *H. bronchisepticus* from the respiratory tract of a number of dogs, cats, rabbits and guinea-pigs. He regarded the organism as a primary cause of canine distemper—a view that has since been disproved (see Chapter 88). *H. bronchisepticus* is now known to be a frequent cause of infectious pneumonia in the lower animals, particularly in guinea-pigs (Smith 1913). It is present often in pure culture in the bronchi. Injected intraperitoneally into guinea-pigs in a dose of 0·5 ml. of a 24-hours' broth culture it causes death in 24 to 48 hours; post mortem there are small hæmorrhages on the peritoneum, and a viscid translucent exudate forming pseudomembranes on the liver, spleen, and the less mobile parts of the intestine; the bacilli are easily recovered from the peritoneal cavity, but with difficulty from the blood, liver or lungs. Subcutaneous injection produces a local lesion only; feeding and inhalation are without effect. The organism is non-pathogenic to mice. It rapidly loses its virulence in culture outside the body.

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CHAPTER 75

UNDULANT FEVER, CONTAGIOUS ABORTION OF CATTLE, AND ALLIED INFECTIONS. TULARÆMIA

UNDULANT FEVER

Ever since the time of Hippocrates a low type of fever characterized by fairly regular remissions or intermissions has been recognized along the Mediterranean littoral. Several names have been applied to it, of which Malta fever and Mediterranean fever are the two best known. In 1887 Bruce reported the discovery of a small micro-organism, which he called *Micrococcus melitensis*, in the spleen of patients dying of the disease. Its injection into monkeys gave rise to a remittent fever, sometimes terminating fatally, and the organism was recovered in pure culture from the liver and spleen. Nearly 20 years later Zammit (1905) of the Malta Board of Health showed that the organism also infected goats. Further work rendered it evident that the goat was in fact the natural host of *Br. melitensis*, and that infection was carried to man by the consumption of raw milk.

In 1918 Evans drew attention to the similarity between the organism isolated from Malta fever and that described by Bang (1897) as the cause of a widespread disease in cattle known as contagious abortion. Her observations were soon confirmed, and in honour of Sir David Bruce the generic name *Brucella* was proposed for the two organisms. The close similarity between *Br. melitensis* and *Br. abortus* suggested the possibility that this latter organism might be pathogenic for human beings. It was not long before Bevan (1921–22) brought evidence to prove the correctness of this surmise. He drew attention to the occurrence in Southern Rhodesia of cases of undulant fever affecting persons who had apparently had no direct or indirect contact with goats. In the areas concerned epizootic abortion of cattle was prevalent, and it appeared probable that infection was being transferred to man by raw cow's milk. Numerous investigators turned their attention to this subject, and before long cases of undulant fever due to *Br. abortus* had been reported from practically every country in the world.

A third causal agent of this disease is *Brucella suis*—an organism originally described by Traum (1914) in the United States. Its natural habitat is the pig. Man becomes infected chiefly by contact with diseased carcasses, and undulant fever due to this organism has therefore a very definite occupational incidence.

These findings have necessitated a reconsideration of the nomenclature of the disease. Since all three organisms give rise to very much the same symptoms in man, and since *Brucella* infections are now known to be widespread over the globe, the term Malta fever is no longer generally applicable. It is being gradually replaced by the term undulant fever, which is free from any geographical connotation. Though this term is satisfactory for febrile infections, it is rather misleading

if used in reference to non-febrile infections with *Brucella* organisms, which are now being recognized as increasingly common. Hence the term Brucellosis has been suggested on analogy with tuberculosis, to apply to all types of *Brucella* infections, febrile or non-febrile, overt or latent, in animals or in man. This term has little to recommend it, since these considerations apply almost equally to diseases caused by other infective agents. We prefer ourselves to use instead the term " Brucella infection."

For the sake of clarity we propose to give a separate description of the epidemiology of undulant fever in man due to the three type organisms. We shall deal first with infections caused by *Br. melitensis*, including in our description the general symptomatology and bacteriology of the disease.

Undulant Fever due to Br. melitensis

Synonyms: Malta fever, Mediterranean fever, Neapolitan fever, Country fever of Constantinople, New fever of Crete, Rock fever of Gibraltar. French: *Mélitococcie.*

Quoting from the classical monograph of Hughes (1897): " Clinically, the fever has a peculiarly irregular temperature curve, consisting of intermittent waves or undulations of pyrexia, of a distinctly remittent character. These pyrexial waves or undulations last, as a rule, from 1 to 3 weeks, with an apyrexial interval, or period of temporary abatement of pyrexial intensity between, lasting for 2 or more days. . . . This pyrexia is usually accompanied by obstinate constipation, progressive anæmia, and debility. It is often complicated with, and followed by, neuralgic symptoms referred to the peripheral or central nervous system; arthritic effusions; painful inflammatory conditions of certain fibrous structures, of a localized nature; or swelling of the testicles." Numerous clinical types are recognized, such as the malignant, the undulatory, the intermittent, and the irregular type. In addition, there are subclinical infections, which can be diagnosed only by bacteriological methods, and infections that may remain latent for weeks or months before flaring up or retrogressing. For a description of these the reader is referred to Marston (1863), who first differentiated Malta fever from other fevers, to Hughes (1897), whose clinical study has never been improved upon, and more recently to Rainsford (1935) and Lisbonne and Janbon (1935). Several complications may occur, beyond those already mentioned, including osteomyelitis, which usually takes the form of localized bone abscesses, bronchitis, and acute inflammation of one or other of the visceral organs (see Michel-Béchet *et al.* 1939).

The commonest symptoms are probably asthenia, fever, muscular and articular pains, nocturnal sweats—often drenching—anorexia, constipation, nervous irritability, and chills or rigors (Taylor and Hazemann 1932).

The incubation period is variable. It may be as short as a week or as long as several months. Most commonly it is 10–30 days. The duration of the illness varies from a few days to over a year, 3 months being the usual time.

Bacteriology.

Bacteriologically, the work of Bruce (1887, 1888, 1893), of Hughes (1893, 1897), and of the Mediterranean Fever Commission (Report 1905–07) showed that the disease was a septicæmia. Working in Malta, Bruce (1893) was able on two occasions to grow the organism from the juice obtained by splenic puncture, and subse-

quent observers showed that, in the early stages of the disease, *Br. melitensis* could frequently be isolated from the peripheral blood. The post-mortem appearances agreed with this interpretation; there was an increase of pericardial fluid, congestion and enlargement of the liver, and great hypertrophy of the spleen. The presence of small areas of congestion in the mucosa and submucosa of the intestine, and the enlargement of the mesenteric glands, the interior of which often consisted of semi-purulent material, were suggestive of an alimentary mode of infection. Cultures taken post mortem from the spleen, liver, and lymphatic glands were positive in 100 per cent. of cases, from the kidney in 85 per cent., and from the heart blood, pericardial fluid and bone marrow in about 50 per cent. (Kennedy 1906). *Br. melitensis* was never found in the saliva nor in the sweat of patients, but it was shown to be quite commonly excreted in the urine. Kennedy (1905), who made a special study of this point, examined no fewer than 1,974 specimens of urine from 61 different patients. In 186 of the samples from 33 of the cases he obtained positive cultures. In a series of 43 cases, in which 20 or more samples from each patient were examined, the organism was isolated from 31. Often the organism could be detected for only 1 day; sometimes it was excreted daily for 2 or 3 weeks. From these figures it may be concluded that, at some time or another, probably at least 75 per cent. of patients suffering from Malta fever pass the specific agent in their urine; this excretion may be sudden and transitory, or it may last for weeks, extending onwards into convalescence.

As the organism is not infrequently found post mortem in the gall-bladder, it is reasonable to suppose that it must be excreted in the fæces. This is probably so, but the technical difficulties of isolating it are considerable. Eyre (1908), however, obtained a positive culture from the colon of a case which came to autopsy.

Outside the body, the organism was found to be fairly resistant; dried on cloth, it remained alive for 17 days, in dust for 44 days, and in sterile tap-water for 20 days (Kennedy 1905).

Epidemiology in Malta.—The epidemiology of undulant fever due to *Br. melitensis* varies in different countries. Since it has been studied most fully in Malta, we may review briefly the findings of the Mediterranean Fever Commission (Report 1905–07) in this island. The maximum incidence rate in Malta was found to occur in July and August. Generally speaking, it varied directly with the atmospheric temperature and inversely with the rainfall. The most susceptible age was between 11 and 30 years, but the disease occurred at any time of life. The upper classes of the civilian population and the officers of the services suffered more heavily than the lower classes and the non-commissioned ranks respectively. During the 33 years 1859–91 the incidence of the disease varied between 269·5 per 1,000 in 1859 and 91·2 per 1,000 in 1888. In spite of this comparatively high rate of morbidity, the mortality rate was low—about 2 per cent. But the duration of the fever and the prolonged disability following its actual termination combined to render it a potent cause of invalidity.

At the time of the Commission's appointment the mode of infection was still unknown. Several ways were suggested, but little evidence was produced in favour of any particular one. Johnstone (1905), who conducted an epidemiological inquiry into the disease, reported that neither food nor drink appeared to have any marked connection with the spread of the fever; nor did dust infection or direct personal contagion play a part. Similarly Davies (1906) concluded that

neither water, milk, nor any other articles of food seemed to be responsible in any way; he favoured a theory of contagion, either direct or through the agency of moquitoes.

It was left to Zammit of the Malta Board of Health, and a member of the Commission on Mediterranean Fever, to indicate the true path of infection. In his first communication (1905) he reported that 5 out of 6 goats which he had examined gave a positive agglutination reaction to *Br. melitensis*. From the blood of two of these animals, he succeeded in cultivating the actual organism itself. Horrocks (1905) rapidly confirmed this, and further demonstrated the frequent presence of *Br. melitensis* in the milk and the urine of apparently healthy goats. Kennedy (1905), who examined the blood serum of 161 goats from 8 different herds, demonstrated the presence of specific agglutinins in no fewer than 84 or 52·2 per cent. of specimens. This high incidence of infection was found to exist not only in the public herds, but in those animals that were kept privately under special care.

As it was evident that *goats' milk* was probably the main source of infection, it was desirable to ascertain on a large scale the proportion of infected animals. For this purpose, Zammit's test was found to be of great value. It consists in observing the presence of agglutinins, not in the blood serum, but in the milk of the goats. The proportion of infected goats judged by this test is definitely lower than that given by the serum agglutination test, since only 60–85 per cent. of the animals showing blood agglutinins also contain agglutinins in their milk. Zammit (1906) examined 710 samples of milk, and obtained 133 positive reactions. From every milk giving a strong reaction he was able to isolate *Br. melitensis* in culture. Horrocks and Kennedy (1906), who made numerous investigations both by the serum and by the milk agglutination test, came to the conclusion that 41 per cent. of the goats in Malta were infected, and that 10 per cent. of those that supplied milk were excreting *Br. melitensis*. It was found difficult to recognize the infected animals on clinical grounds; they did not suffer from a true fever, and though some showed loss of weight, a thinning of the coat, and a short hacking cough, others remained perfectly well and displayed no sign of illness. The amount of milk secreted was likewise no criterion, for the infected animals frequently yielded as much and more than the non-infected ones.

From these investigations it was apparent that the goats of Malta were heavily infected with *Br. melitensis*, and that about 10 per cent. of the milch goats were excreting this organism in their milk. As the majority of milk consumed in the island was goats' milk—cows are few owing to insufficient pasturage—it was easy to see how the disease might be transmitted to human beings.

Steps were taken by both the Navy and the Army, in 1906, to stop the supply of goats' milk to the troops. The result was most striking. Within a year the disease had been practically eradicated, only a few easily explained cases occurring. Amongst the civil population, such radical steps could not be taken; the gradually decreasing incidence observed during the following years was largely due to the killing off of many of the infected goats (Table 162).

This method, however, afforded no permanent protection, and the disease continued to exact a heavy toll. Of recent years it has been increasing in extent and severity among the civil population. There has, in fact, been a steady rise from 1926, when there were 596 cases with 27 deaths, till 1934, when there were 1,909 cases with 88 deaths.

TABLE 162 (taken from Eyre 1908, 1912).

	Civil.		Navy.		Army.	
	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
1901 . . .	642	54	252	3	253	9
1902 . . .	624	45	354	2	155	6
1903 . . .	589	48	339	6	404	9
1904 . . .	573	59	333	8	320	12
1905 . . .	663	88	270	7	643	16
1906 . . .	822	117	145	4	163	2
1907 . . .	714	78	12	0	9	1
1908 . . .	502	?	6	—	5	?
1909 . . .	456	?	10	—	1	?
1910 . . .	318	?	3	—	1	?

Consumption of goats' milk was not the only method of infection, though it was undoubtedly the most important for the troops and naval ratings on the island. Contamination of the skin or mucosæ with infective material—particularly common in laboratory workers—afforded another method. Occasionally infection appeared to be insect-borne, or transmitted by sexual congress (Eyre, McNaught, Kennedy, and Zammit 1907).

The history of Malta fever is instructive. In the first place, it affords an excellent example of a disease whose incidence was not immediately affected by the discovery of the causative agent. In order to prevent a disease, it is not necessary to know the causative agent, so long as we know the way in which it spreads. Once this is known, the steps necessary for limiting it may be considered. In some cases, as in that of milk-borne disease, the steps may be relatively simple ; stop the supply of milk, or insist on its adequate heat treatment, and the infection can no longer be transmitted to human beings. In other cases, notably the respiratory diseases, the problem is more difficult. No satisfactory method for preventing the inhalation of infected material has yet been devised ; and until this proves possible, measures against these diseases will be more profitably directed towards increasing the host resistance than to interfering with the spread of infection.

The second point of interest is that, in an epidemiological inquiry, it is harder to assess the importance of a given factor when it operates more or less uniformly throughout a population, than when it is distributed in such a way as to affect different sections unevenly. Both the epidemiologists who investigated the disease in Malta (Johnstone 1905, Davies 1906) reported that there was no evidence to suggest that infection was conveyed by food. Both of them overlooked the fact that a disease spread by an article of almost universal consumption, such as goats' milk, would show little preference for any one class of the population, if the contamination of the milk were widespread and frequent, rather than local and occasional. The clue to the problem was furnished by the bacteriologist. It was this second discovery, that a large proportion of goats contained agglutinins in their blood for *Br. melitensis*, which complemented the first discovery, that of the organism itself, and pointed out the way by which the spread of the disease might be checked.

(For an excellent account of the epidemiology of Malta fever in the Royal Navy, see Dudley 1931.)

Epidemiology in Other Countries.—The next most important bacteriological and epidemiological study of the disease has been made by Taylor and his colleagues in the South of *France*, working at the Undulant Fever Centre established at Montpellier by the Rockefeller Foundation (see Taylor and Hazemann 1932, Taylor, Lisbonne, and Roman 1932, Taylor, Lisbonne, and Vidal 1935, Taylor *et al.* 1938*a*). The disease was found to be widespread in the south-east of France, where sheep and goats are common and cattle are relatively few. The incidence in males was 2–3 times that in females, and was highest in the 15–45 age group. The occupational incidence was very striking, the majority of the cases occurring in the agricultural community, especially among those engaged in rearing sheep and goats. The seasonal prevalence was at its maximum in the spring, corresponding to the time of lambing and abortion. Though the consumption of goats' milk, and of fresh cheese made from goats' or ewes' milk, undoubtedly played some part in infection (see Carrieu and Lafenêtre 1932, Veloppé and Jaubert 1935), the evidence on the whole suggested that infection most frequently resulted from direct contact with infected animals and manure. In a population, however, living under very primitive conditions, and exposed to many sources of infection, it was difficult to ascertain with certainty which was the most important route by which the organisms gained access to the body. The very interesting observation was made (Taylor, Vidal and Roman 1934) that cows in close contact with sheep and goats might become infected with *Br. melitensis*, excrete this organism for months in the milk, and give rise to undulant fever in human beings consuming it in the raw condition.

The disease affects several other countries, including Italy, Spain, Greece, Transcaucasia, Algeria, Tunis, Palestine, Arabia, India, South Africa, China, and various parts of South America. Like Malta, Corsica and Sicily are seriously affected. In this country, owing to the absence of *melitensis* infection in sheep and goats, disease due to this organism does not occur. In the United States there is a certain amount of undulant fever due to *Br. melitensis* in the goat-raising areas, but most of it is due to *Br. abortus* and *Br. suis*.

Undulant Fever due to Br. abortus

In its general symptomatology and bacteriology this disease resembles that caused by *Br. melitensis*. Though, as will be pointed out later, the ability of *Br. abortus* to give rise to undulant fever is less than that of *Br. melitensis*, once the typical disease has developed, it seems to pursue its course more or less independently of the type of the causative organism. The incubation period is very variable, ranging from a week or so to 6 months or more (see Hardy, Frant and Kroll 1938). The average duration of the disease is 3 months, and the case mortality about 2 per cent. Besides typical cases, however, a considerable number of so-called subclinical infections occur, characterized by only slight pyrexia and by a variety of different symptoms. Evans (1934) draws attention to the frequency in these patients of exhaustion, insomnia, irritability, and subjective disturbances, leading to a diagnosis of neurasthenia. Sometimes there is an acute influenza-like illness lasting for not more than a week, while at others a low intermittent fever may persist for months or even years. Acute abdominal symptoms are not uncommon, and in several patients an appendicectomy or cholecystectomy has been performed before the real nature of the disease was recognized (Simpson 1930). Chronic infections may occur (see Evans *et al.* 1938, Calder 1939).

Infection during pregnancy is sometimes, but not often, followed by abortion. The organism may be isolated from the placenta and uterus of the mother and from the stomach contents of the fœtus (see Kristensen and Holm 1929, Spengler 1929, Simpson 1930, Alessandrini and Pacelli 1932, Habs 1933*a*, Lauda 1934, Menzani and de Zanche 1934).

Frequency.—Though the disease is widespread throughout the world, its frequency is difficult to assess. As in tuberculosis, the reported cases may bear very little relation to the real incidence of infection. The reason for this is partly that the disease is still unknown to many practitioners, partly that it is impossible to diagnose with certainty except by bacteriological methods, and partly that in several countries, including Great Britain, the disease is not reportable. The so-called reported cases often refer to mere collections by private persons. The nearest approach we can make to ascertaining the incidence of the disease is by indirect methods, particularly by noting the proportion of sera from patients with undiagnosed pyrexia which agglutinate *Br. abortus* to a significant titre. In some countries, such as Denmark, where serological examination of pyrexial patients is common, and where all sera are examined under standard conditions in one central laboratory, it is easy by this means to obtain a fair estimate of the frequency of active infection with *Br. abortus*. In other countries, such as our own, where serological examination, especially in rural areas, is more restricted, and where sera are examined in multiple scattered laboratories, collection and integration of the data are very much harder. With reservations to be made later for certain occupational classes which are exposed to frequent infection with *Br. abortus*, a titre of 1/80 or over in febrile patients can be regarded as probably indicative of active infection. Having ascertained the proportion of Widal sera agglutinating *Br. abortus*, the probable number of cases of infection can be calculated, either directly if the total number of Widal sera examined in the whole country is known, or indirectly by a method described elsewhere (Wilson 1932*a*). The results obtained (see Table 163) are essentially estimates, and probably err on the conservative side. (For references to figures used, see Kristensen and Holm 1929, Holl 1930, Weigmann 1931, Bijl and v.d. Hoeden 1931, Süpfle and Hofmann 1932, Bach 1932, Fried 1932, Grumbach and Grilichess 1932, Messer 1932, Dettling 1932, Wade 1933, Hall 1933, Russ 1934, Kalbfleisch and Kalbfleisch 1935.) It will be

TABLE 163

PROPORTION OF WIDAL SERA AGGLUTINATING *Br. abortus* TO 1/80 OR OVER, AND ESTIMATED ANNUAL NUMBER OF UNDULANT FEVER CASES ABOUT 1930.

(Data compiled from several papers in different countries; for references, see text.)

Country.	No. of Sera Examined.	No Positive.	Percentage Positive.	Estimated Annual Number of Undulant Fever Cases.	No. of Cases per 1,000,000 Population.
Great Britain . . .	3,175	101	3·18	440	11
Germany	9,397	323	3·44	600	10
Austria	9,693	177	1·83	50	8
Switzerland	1,503	91	6·1	340	84
Holland	4,500	50	1·11	90	12
Denmark	4,623	500	10·82	500	147
Sweden	—	—	—	120	20

seen that the incidence of the disease, calculated per million of the population, is much the same in Great Britain, Germany, Austria, Holland, and Sweden, but that it is considerably higher in Switzerland and Denmark. In the United States undulant fever is known to be very prevalent, but as some of the cases are due to infection with *Br. suis*, and the antigenic structure of this organism is closely related to that of *Br. abortus* (see Chapter 34), figures based on agglutination results are not strictly comparable with those just given. There is evidence to suggest that the different incidence in different countries is dependent to some extent on the frequency of contagious abortion in cattle, and the degree of exposure of the human population, though other factors undoubtedly play a part (Henricsson 1932, Olin 1935). Some of the difference is almost certainly more apparent than real, and is due to the much better diagnostic facilities available in certain countries than others. Further data on the incidence of the disease may be consulted for Great Britain (Menton 1937), Yugoslavia (Simitch and Djourichitch 1936), South Africa (Campbell and Greenfield 1937) and Canada (Dolman and Hudson 1938).

Latent Infections.—Besides definite clinical disease, *Br. abortus* gives rise to a considerable amount of latent infection. Examination of Wassermann sera in this country and in Germany shows that about 1·5 per cent. agglutinate *Br. abortus* to a titre of 1/40 or over. The titre is generally less than 1/160, and high titres, such as those yielded by the majority of sera from patients with undulant fever, are uncommon. A certain proportion of these sera are derived from patients actually suffering from the disease. The majority, however, come from persons with no clinical evidence of undulant fever. The probability is that these agglutinins are due to latent infections with *Br. abortus*. This interpretation is supported by two facts. The first is that agglutinins, even in a titre of 1/20, are rarely present in the sera of persons who do not drink raw milk or cream, and who are not exposed to contact with infected animals. The second is that agglutinins are present in quite a high proportion of persons who drink considerable quantities of infected raw milk or cream, or whose duties bring them into close contact with infected animals (Table 164). (For references to figures used, see Lentze 1930, Martin and Myers 1931, Maclean 1932, Wilson 1932*b*, Starr and Maxcy 1933, Molinelli, 1933, 1934, Hermann *et al.* 1934, v. Berkessy and Simon 1934, v. Berkessy 1935, Zimmermann 1935, Rossi 1935) (see also Beattie 1938).) The figures given in the table include the United States and the Argentine, in both of which countries there is some infection with *Br. suis*, but it will be seen that in all countries about 10–20 per cent. of persons in these special occupational classes contained agglutinins to *Brucella*. Investigation showed that some of these persons were suffering from undulant fever at the time of examination, or had recently recovered from it, but most of them had no symptoms indicative of active infection. An exception should perhaps be made for veterinary surgeons, several of whom suffered from an allergic condition, which manifested itself in the form of a rash on the arm developing after " cleansing," *i.e.*, evacuation of the cow's uterus (see Huddleson and Johnson 1930, Haxthausen and Thomsen 1931, Wilson 1932*b*, Thomsen 1937*c*).

The conclusion seems to be that infection with *Br. abortus* resembles in many respects infection with the tubercle bacillus. Both organisms have a fairly high degree of infectivity enabling them to establish themselves, at least temporarily, in the tissues. Neither of them, however, has a high pathogenicity, so that in the majority of persons the infection remains latent or retrogresses. Only in a small proportion of infected persons are the conditions favourable for the production

TABLE 164

PROPORTION OF SERA AGGLUTINATING *Br. abortus* TO 1/40 OR OVER IN PERSONS EXPOSED TO INFECTION.

(Data compiled from several papers in different countries; for references, see text.)

Occupational Class.	Country.	No. of Sera.	No. Positive.	Percentage Positive.
Milk and Farm Employees	Germany	220	31	14·1
	Hungary	63	10	15·9
	New Zealand	104	17	16·4
	Argentine	136	16	11·8
Slaughterers	Great Britain	206	27	13·1
	Hungary	93	21	22·6
	U.S.A.	452	62	13·7
	Argentine	1,776	191	10·8
Veterinarians	Great Britain	63	13	20·6
	France	28	7	25·0
	Denmark	94	22	23·4
	U.S.A.	715	92	12·9
	Argentine	110	29	26·4

of clinically detectable disease. It is probable that latent infections often lead to latent immunization, thus explaining why undulant fever does not occur more often than it apparently does in veterinarians. There is also evidence to suggest that persons who are exposed to heavy infection for the first time, or after a long interval of freedom from exposure, are more likely to develop the disease than those who are exposed to mild infection more or less continuously. Garrod (1937) has drawn attention to the susceptility of *Br. abortus* to the bactericidal action of the gastric juice, and suggests that this may be one reason why undulant fever is not commoner than it is. It would be idle to pretend, however, that we have any exact knowledge of the factors determining the course of infection in any given individual, and those who deny the pathogenicity of *Br. abortus* for man because not every exposed person develops the disease, must be wholly unaware of the complexity of the equilibrium existing between host and parasite. The possibility that strains of *Br. abortus* vary in virulence must be considered, but so far there is no evidence to support it (see Birch and Gilman 1935, Olin 1935).

Mode of Infection.—The mode of infection varies in different countries and in different parts of the same country. The main source is obviously cattle, and man becomes infected by consumption of raw milk or cream, or by contact with infected animals, either alive or dead. Generally speaking, the town population is exposed to infection from milk, while the country population is exposed to both milk-borne and direct contact infection.

Numerous records in different countries show that a considerable proportion of samples of raw milk coming from infected herds contain living *abortus* bacilli. In Great Britain about 20–30 per cent. of herd samples are infected. Experience has shown that *consumption of raw infected milk* is attended by a certain risk of contracting the disease, whereas consumption of the same milk after pasteurization or other effective heat treatment is harmless. In London, where about 98 per cent. of the milk is heat-treated, and in the large cities of the United States

where most of the milk is pasteurized, undulant fever is practically unknown. The cases that do occur are mostly among those who drink Certified or other types of raw milk, either at home or on a visit to the country.

Several careful studies made in institutions and small communities supplied with infected raw milk have shown that a considerable proportion of the population develop serum agglutinins, that a small proportion suffer from subclinical and clinical forms of undulant fever, while, in a larger proportion, the infection remains completely latent. Removal of the infected animals, or pasteurization of the milk supply, is followed by cessation of clinical illness and by a gradual disappearance of agglutinins from the sera of latently infected persons (see King and Caldwell 1929, Hasseltine and Knight 1931, Sasano, Caldwell and Medlar 1931, Dooley 1932, Johns, Campbell and Tennant 1932, Wilcox 1932, Carpenter and Boak 1933, Welch and Mickle 1933, Hall and Learmonth 1934, Cameron and Wells 1934, Stone and Bogen 1935, von Engel 1938, Elkington *et al.* 1940, Cruickshank and Stevenson 1942).

It may be pointed out here that alimentary infection is almost always due to the consumption of raw milk or cream. Butter and cheese, except when made from untreated milk and consumed within a week or two, are probably inoffensive, since the organisms die out very rapidly as the result of lactic fermentation (Mazé and Césari 1931, Caronna 1936, Taylor *et al.* 1938*a*). Moreover, a large proportion of manufactured butter is made from pasteurized cream in which the organisms are destroyed (see Smith, J., 1934*a*, Pullinger 1935). One curious laboratory outbreak, possibly associated with defective plumbing, is on record (Huddleson and Munger 1940).

Contact infections are mainly occupational, occurring in towns among slaughterers, and in the country among the farming population. Since the latter class, in particular, is usually exposed to infection from raw milk, the parts played by the two sources of infection are very difficult to determine. In countries with a large agricultural population, such as Denmark, it follows that the incidence of undulant fever is greater in rural than in urban districts, whereas in countries such as our own, where about 80 per cent. of the population is concentrated in towns, the reverse holds true. Contact infection is mainly from cattle, but horses and even dogs may at times constitute the source of infection (Menzani 1932). In both town and country the brunt of the disease is generally borne by *adult males*. The incidence is usually highest in the 15–45 age group, and is two to three times as high in males as in females. The age and sex incidence in country districts seems readily understandable, because of the predominance of male workers in contact with cattle, but in towns it is more puzzling. There is some evidence that in towns latent infections are commoner in women than in men, but that overt infections are commoner in men than in women (Harrison and Wilson 1928). The suggestion is that the greater consumption of raw milk in females confers on them an immunity which is often lacking in males, so that when the latter are exposed to infection, they prove more susceptible. Much the same argument has been used by Henricsson (1932) to explain why, in Sweden, more cases occur in the town than the country, namely, that the rural becomes better immunized than the urban population as the result of more frequent exposure to infection. Some workers (Hardy *et al.* 1931) have found that in towns males and females are affected more evenly than in the country, but there are numerous other observations to show that even in towns the adult male population provides the bulk of the cases.

Laboratory infections, though less common than with *Br. melitensis*, are by no means infrequent, and probably occur through contamination of the abraded skin or the conjunctiva with infective material (see Meyer 1933, Meyer and Eddie 1941). The incidence in veterinary surgeons seems to be definitely higher than in the general population, but the information available from different countries is rather conflicting (see Spengler 1929, Huddleson and Johnson 1930, Knoth 1930*a*, Makkawejsky and Karkadinowsky 1930, Thomsen 1932*a*, Wilson 1932*b*, Herrmann *et al.* 1934, Khaum and Liedl 1935). Quite a high proportion of those engaged in "cleansing" develop the peculiar skin rash to which allusion has already been made. Both infants and children suffer from the disease, but very much less frequently than adults.

(For general epidemiological studies and reviews of the disease, in addition to those already quoted, see Simpson and Fraizer 1929, Dalrymple-Champneys 1929, 1931, 1934, 1935; Darsin 1930, Wallace 1930, Atwood and Hasseltine 1930, Zeller 1931, Tebbutt and Marsh 1931, Cruickshank and Barbour 1931, Smith 1932*a*, Schittenhelm 1932, Hardy, Jordan and Borts 1932, 1936, Mackie 1933, Habs 1933*b*, 1935, Steyrer 1934, Meyer *et al.* 1934*a*, Meyer 1938, McNabb 1934, Report 1934, Giltner 1934, Beattie, Smith and Tulloch 1935, Wilson 1935, Taylor *et al.* 1938*a*, Huddleson 1939.)

Undulant Fever due to Br. suis

Probably the first diagnosed case of this disease was that described by Keefer (1924) in 1922 in the United States, though it was some time before the identity of the causative organism was realized. Clinically and bacteriologically the disease is similar to undulant fever caused by the *melitensis* and *abortus* types. Like *Br. melitensis*, *Br. suis* can be demonstrated more frequently in the blood stream than *Br. abortus*—possibly because of its greater ease of cultivation.

Epidemiologically, this disease is very much less extensive than the types already described. It is essentially an occupational disease, occurring among slaughterers and packers who handle the infected carcasses of pigs. The studies of Hardy and his colleagues (1931, 1932), of Huddleson, Johnson, and Hamann (1933*a*), and of Heathman (1934) in the middle western states of North America, have shown that a small amount of overt and a large amount of latent infection occurs in these workers. The organism apparently gains access through the abraded skin. Adult males are almost solely affected (Hasseltine 1930). Occasionally *Br. suis* infects cattle, and milk-borne outbreaks of undulant fever, without any special occupational incidence, are likely to occur (see Beattie and Rice 1934, Horning 1935, Borts *et al.* 1943). Brucella infection of pigs is far less common than contagious abortion of cattle. In consequence undulant fever due to *Br. suis* has hitherto been restricted mainly to the middle-west of North America, to Brazil, and to the Argentine.

Diagnosis of Undulant Fever

The occurrence of subclinical and atypical infections renders the clinical diagnosis of undulant fever peculiarly difficult, and all observers are agreed on the fact that large numbers of cases are missed. The final diagnosis can be made only by bacteriological examination. In practice it is well to regard every case of pyrexia of undiagnosed origin as possibly due to *Brucella* infection, and to bear this disease

in mind during the investigation of acute and chronic inflammatory conditions in which the diagnosis remains doubtful. Help is sometimes afforded by a blood count, which usually shows a secondary anæmia, a mild leucopenia, and a relative lymphocytosis.

Blood Culture.—In the febrile stages of the disease, an attempt should be made to isolate the causative organism from the blood stream.

The patient's blood should be withdrawn while the temperature is elevated, and preferably during the rise of a pyrexial wave. It should be distributed in 5–10 ml. quantities into two flasks containing 20–100 ml. of glucose broth, serum broth, Bacto tryptose broth, or liver extract broth. One of the flasks should be incubated aerobically, the other in an atmosphere in which 10–25 per cent. of the air has been replaced by CO_2. Subcultures should be made every 3–5 days on to a solid medium, and these should be incubated in the same atmosphere as the parent culture. Growth is often slow, and no culture should be discarded in less than 4–8 weeks. To guard against contamination, 1/20,000 Victoria blue (Hauptmann 1937), or 1/700,000 crystal violet (Huddleson 1939), may be added to the culture. Two ml. of the patient's blood may be inoculated intraperitoneally into guinea-pigs (Poston 1938). (For alternative methods of blood culture, see Elkeles and Fried 1932, Rainsford 1933, Hauptmann 1935*a*, Stewart *et al.* 1935.)

Though blood culture is positive in a high proportion of *melitensis* and *suis* infections, not more than 10–20 per cent. of *abortus* infections prove positive by this method. In *melitensis* infections the organism can often be isolated from the urine, provided repeated samples are examined (Kennedy 1905). Occasionally *Br. abortus* can be demonstrated in the bile (Amoss and Poston 1930, Leavell and Amoss 1931*a*) stools (Smith 1932*b*, Beattie *et al.* 1935, Goldstein *et al.* 1936) and tonsil (Carpenter and Boak 1932, Poelma and Pickens 1932).

Agglutination Test.—The most generally useful method is the agglutination test, first introduced by Wright and Smith in 1897.

Final dilutions of the serum should be put up ranging from 1/20–1/5120, and the tubes should be incubated for 18 hours in a 37° C. or 50° C. water bath. Though there is a considerable degree of cross-agglutination between *Br. abortus* and *Br. suis* on the one hand, and *Br. melitensis* on the other, it is advisable to use as agglutinating antigens strains corresponding to the prevailing type of infection. In countries such as Great Britain, Germany, Denmark, Sweden, Canada, and New Zealand, where practically all infections are due to the *abortus* type, this organism alone is necessary. Similarly in countries such as Malta, Corsica, Sicily, Southern Italy, Algeria, Tunis, Greece, and Palestine, where nearly all infections are due to the *melitensis* type, this organism suffices. But in countries or areas where two or three types of infecting organism are known to occur, such as France, Northern Italy, the United States, and parts of South America, it is advisable to use both a *melitensis* and an *abortus* antigen. Great attention must be paid to the preparation of the agglutinating suspension. Only strains that are absolutely smooth, as judged by the thermoagglutination and acriflavine tests, should be used. Routine suspensions should be made up from a number of strains. The organisms should be grown on liver extract or glycerol agar for 2–3 days, washed off with saline, and heated to 55° C. for 1 hour. The suspension should be standardized to match an opacity tube containing 1,000 million *Bact. coli* per ml., and preserved with 0·2 per cent. formol or 0·5 per cent. phenol. Suspensions prepared in this way will generally remain satisfactory for 3 months, but the moment signs of commencing stringiness appear, they should be discarded. For survey work, where large numbers of sera have to be examined, the slide method of agglutination may be used (see Huddleson and Abell 1928, Huddleson 1932, 1939, Welch and Mickle 1933).

The interpretation of the agglutination test is not always easy, and demands a knowledge of the clinical history and the occupation of the patient. Very much the same considerations affect the interpretation of this test as of that used in the diagnosis of enteric fever, and the reader may well refer back to Chapter 69 in which the relevant factors are discussed in some detail. The propositions we lay down must not be regarded as more than suggestions, applying particularly to this country. Each of them will necessarily be subject to exceptions. (1) A titre of 1/80 or less, in the absence of clinical symptoms, is indicative either of a latent *Brucella* infection, or of a past infection—not necessarily attended by definite disease. (2) A titre of 1/80 or over, in the absence of clinical symptoms or of a recent pyrexial attack, is suggestive of frequent infections, usually occurring in persons drinking large quantities of infected raw milk or exposed to contact with infected animals or carcasses. (3) A titre of 1/80 or over in the presence of pyrexia and other symptoms of disease, occurring in a person whose occupation or habits do not expose him to special risk, is very suggestive of active infection with a member of the *Brucella* group. In persons belonging to the occupational classes referred to, in whom a latent infection is not uncommon, a titre of 1/80 is too low to be of diagnostic significance. On the other hand, a titre of 1/1000 or over is rarely met with except as the result of an active infection, and may usually be regarded as evidence of undulant fever. (4) A titre of 1/20–1/80, in the presence of clinically undulant fever, may likewise be considered as practically diagnostic of this disease. (5) The complete absence of agglutinins from a patient's serum does not exclude the diagnosis of *Brucella* infection. Cases are on record in which a positive blood culture has been obtained in the presence of a negative agglutination reaction (see Gilbert and Dacey 1932, Heathman 1934, Taylor *et al.* 1938*a*). Agglutinins are generally present in a suggestive titre by the end of the 2nd week, and in frank cases of undulant fever they generally rise to a titre of 1/640 or more. After the attack is over they tend to fall fairly rapidly, and may sink to a low level within 3 months. There is evidence that in chronic cases they may fall even during the period of active infection, so that too much stress must not be laid on a negative agglutination reaction in patients whose pyrexia has lasted for some months.

Attention must be called to the not infrequent occurrence of a prozone, sometimes extending to even 1/640 in high-titre sera. For this reason a wide range of dilutions should always be put up (see Spencer 1930, Priestley 1931, Hirsch 1935).

Several observers have stated that the agglutinin titre to *Brucella* may be high in patients suffering from enteric fever, tuberculosis, and other febrile conditions. The evidence for this statement is not very satisfactory. Apart from the anamnestic reaction, which is not likely to account for more than a slight raising of the titre (see Amoia 1933), the most common cause of non-specific agglutination is the use of a suspension made from strains that are not absolutely smooth. Such strains are often agglutinated by normal serum, and apparently even more often by the sera of febrile patients. Their use in the past has been very common and has given rise to much confusion in the literature. Provided smooth strains only are used, it is justifiable to conclude that an elevated titre to *Brucella* is indicative of infection—latent, active, or past—with an organism of the *Brucella* group. (For recent references see Bayne-Jones 1930, Ranque and Senez 1932, Magliulo 1933, Morellini 1933, Sanfilippo 1933, Wilson 1930, 1934, Mohr 1935, Hauptmann 1935*b*.) We may add that there is no evidence that circulating agglutinins result from the alimentary absorption of milk containing antibodies (see Boak and Carpenter 1929, Peterson 1935).

The type of infecting organism may often be determined by use of the quantitative agglutinin absorption test, described in Chapter 34, carried out on the patient's serum (see Habs and Sievert 1935).

The *complement-fixation reaction* may be used to confirm the agglutination test, or in suggestive cases when the latter test is negative. Complement-fixing bodies are said to appear later in the serum than agglutinins, and to persist longer (see Sasano, Caldwell, and Medlar 1931, Morales-Otero and Monge 1932, Laun and Heide 1934). A precipitation reaction has also been described (Schlesmann 1932).

Brucellin Test.—In 1922 Burnet drew attention to an allergic skin test, performed by the intradermal inoculation into the arm of 0·05–0·1 ml. of a filtrate of a 20-day-old broth culture of *Br. melitensis* (*melitin*) or *Br. abortus* (*abortin*). A positive reaction is characterized by the appearance in 6 hours of a slightly raised, sometimes tender, œdematous plaque, 2–6 cm. in diameter, distinguished in colour from the surrounding skin. The reaction may disappear within 48 hours, or a central nodule may develop lasting for several days. Pseudo-reactions generally appear rapidly, and disappear within 24 hours. We have no space for an adequate discussion of this test. Its interpretation and value are rendered difficult by the fact that numerous preparations of brucellin (abortin or melitin) are in use. The potency of these is subject to considerable variation, and some of them give rise to reactions in normal persons. Not until a standard preparation and standard dose are used, will it be possible to assess the value of this test, Generally speaking, however, the reaction resembles the tuberculin test in denoting the occurrence of infection, without giving any clear indication as to its activity. In undulant fever it may not become positive for some weeks, but subsequently it remains positive, often for years. Its chief value will probably be found to lie in the diagnosis of chronic cases with a negative agglutination reaction, and in survey work for detecting the prevalence of *Brucella* infection (see Giordano 1929, Simpson 1930, Levin 1930, Leavell and Amoss 1931*b*, Straube 1932, Dubois and Sollier 1932*a*, Yeckel and Chapman 1933, Rainsford 1933, Huddleson *et al.* 1933*b*, Heathman 1934, Arbatskaia and Moroskin 1934, Goldstein 1934, Sarnowiec 1934, Olin 1935, Favorite and Culp 1935, Taylor, Lisbonne and Vidal 1935, Chiucini 1935). More recently Huddleson (1939) has described the preparation of a *Brucella* nucleoprotein extract called *brucellergin*, which he recommends for the skin test; 0·1 ml. of a 1/2,000 dilution is injected intradermally. A negative reaction is said virtually to exclude *Brucella* infection; a positive test indicates that the patient has been infected in the past or is infected at the moment (Gould and Huddleson 1937). Whether brucellin or brucellergin be used, it is important to remember that the injection of either may be followed by the appearance of circulating antibodies.

The difficulty of interpreting the skin reaction has led Huddleson, Johnson, and Hamann (1933*b*) to introduce the so-called *opsono-cytophagic test.* Briefly, this consists in incubating the citrated blood of the patient with a *Brucella* suspension for 30 minutes at 37° C., making films, and counting the number of organisms in 25 leucocytes. It is said that a weak or negative reaction in conjunction with a negative skin test is evidence of susceptibility, that a negative reaction in conjunction with a positive skin reaction points to active *Brucella* infection, and that a positive reaction to both tests is indicative of immunity. There is some discrepancy of opinion between different workers on the value of this test (see Meyer *et al.* 1934*b*, Veazie and Meyer 1935, Keller *et al.* 1936, Gould and Huddleson 1937, Keller, Pharris and Gaub 1938, Menefee and Poston 1939, Evans 1939, Castaneda *et al.* 1942, Tovar 1944).

For *post-mortem diagnosis*, cultures should be made from the spleen, liver, kidney and mesenteric glands, and incubated aerobically and in 10 per cent. CO_2. Alternatively, the ground-up organs may be suspended in broth and injected intramuscularly into guinea-pigs.

Prophylaxis and Treatment

The prevention of the disease in human beings consists ideally in the complete abolition of contact, direct or indirect, with infected animals or their products. Where infection is chiefly milk-borne, this may be comparatively easy. Either complete avoidance of milk, or its consumption only after pasteurization, boiling or sterilization, has been found in practice to be highly effective. Where, however, infection occurs mainly by contact, prevention becomes much more difficult. Vaccination of specially exposed persons may be tried, but there is as yet little evidence to show how far this method is likely to be successful.

Treatment of the disease itself is very unsatisfactory, and in spite of all measures the fever may continue for months or even years. The oral administration of dyes has been recommended by Leavell, Poston, and Amoss (1930). Several workers have claimed success by the use of intramuscular inoculation of autogenous or stock *Brucella* vaccines, given in a dose sufficient to evoke a smart febrile reaction and repeated every 3–7 days (see Schilling *et al.* 1931, Poppe 1933, Bianchi 1934). Di Guglielmo (1933) is supported by numerous observers in his claim for the therapeutic efficacy of intravenous administration of a *Brucella* vaccine. After one or two doses of about a million organisms to test the patient's sensitivity, doses increasing from 5 to 100 million are given every 3–4 days, till the temperature finally falls. Usually 7 injections, lasting over a month, are said to be required to bring about this end. Much the same results appear to be obtainable by protein shock therapy. Huddleson and Johnson (1933) and Huddleson (1939) in the United States, and Debono (1935) in Malta, have both reported favourably on the intramuscular injection of brucellin. Our own experience with this preparation has also been favourable.

It is advisable to test the patient's sensitivity by first inoculating 0·1 ml. into the skin. If there is little or no systemic reaction within 24 hours, treatment may be begun by the intramuscular injection of 0·5–1·0 ml. Further injections may be given at 3- or 4-day intervals, the dose being gauged so as to cause a brisk febrile reaction each time. In some cases one or two doses suffice to effect cure, but more often three or four are needed. If no reaction follows the first intramuscular inoculation of 1·0 ml., treatment will almost certainly prove useless. If the preliminary intracutaneous inoculation causes a systemic reaction, small doses only should be given, preferably by the intradermal route.

Serum treatment has been advocated (Hilgermann 1935), but judged by animal experiments its effectiveness is doubtful (Mitchell *et al.* 1935, Gwatkin 1938). Sulphonamide treatment has been recommended by a number of workers on the basis of one or two apparent cures, but observations on guinea-pigs hold out little promise of its success (Wilson and Maier 1939, 1940, Hamann and Huddleson 1939, Priestley 1940).

BRUCELLA INFECTION OF SHEEP AND GOATS

Reference has already been made, under the section dealing with undulant fever due to *Br. melitensis*, to infections of sheep and goats with this organism, and to the diagnosis of the disease by examination of the blood serum and milk serum for agglutinins. The disease exists particularly in countries along the Mediterranean littoral, in South Africa, and in some parts, notably Arizona, of the

United States. When first introduced into a herd, particularly during pregnancy, infection is frequently followed by a considerable number of abortions, but unless fresh animals are added, it dies down, and subsequent abortions are uncommon. The infection, however, usually becomes chronic, and may persist for months or years. In this stage it is often difficult to diagnose. The agglutination reaction may be negative. Some help is afforded by the intradermal melitin test. A suitable preparation, preferably a bacterial extract such as that described by Huddleson and Johnson (1933) or Taylor, Lisbonne, and Vidal (1935), is inoculated into one of the skin folds between the base of the tail and the margin of the anus. Readings are made after 24 and 48 hours. A positive reaction is characterized by œdema. Generally speaking, it may be said that a positive skin test indicates that the animal has been or is infected, while a positive agglutination test indicates that the infection is active. At post-mortem examination the organisms can be isolated most easily from the udder and the supramammary lymphatic glands. During life the organisms are often excreted in enormous numbers in the milk and the urine, while after abortion the vaginal discharge may remain highly infective for some days. Infection probably occurs mainly by contamination of the skin and mucosæ, though possibly the alimentary route may play some part. It seems to be more persistent in goats than in sheep. In both animals the young are less susceptible than the sexually mature.

Experimentally, the disease can be reproduced in both sheep and goats by feeding and by cutaneous, subcutaneous, intravenous, and intraperitoneal inoculation with virulent cultures of *Br. melitensis*. The disease so produced is often afebrile and, in spite of a septicæmia, symptoms may be lacking. Many of the animals, however, are unthrifty; their coats become thin; their weight diminishes; and a short hacking cough develops. Mastitis and arthritis are not uncommon; male animals may suffer from orchitis, while females, if infected during pregnancy, may abort. Death rarely occurs. Bacteriologically, the organisms are found in the blood for a variable time after infection. They then disappear, first from the peripheral blood and most of the viscera, then from the spleen and kidneys, then from the superficial lymph glands, and last of all from the mammary glands. The milk does not usually become infective for 2–3 months after inoculation or feeding. Agglutinins, sometimes reaching a high titre, are demonstrable in the blood serum, usually within a fortnight or so of infection (see Shaw 1906, Horrocks and Kennedy 1906, Zammit 1906, Eyre *et al.* 1907).

Experimentally, disease can also be produced in sheep and goats by *Br. abortus*, though natural infections with this organism seem to be rare. Bang (1897) induced abortion in a pregnant ewe by intravaginal injection of a pure culture of *Br. abortus*. Intravenous inoculation gave rise to uterine inflammation. Zwick (1910) found that both feeding and intravenous inoculation were successful in establishing infection in sheep. Infected ewes may go to term, yet the specific organisms may be found in large numbers in the vaginal discharge. Intravenous inoculation of *Br. abortus* into goats may give rise to abortion (Reinhardt and Gauss 1915). Zwick and Krage (1913) found that goats injected subcutaneously or intravenously might excrete the organism in their milk 24 hours later; the excretion was sometimes maintained for 8 weeks or more. Goats are also susceptible to conjunctival infection with *Br. abortus* (van der Hoeden 1933).

The control of the disease is beset with difficulties of an economic and social nature. Prophylactic vaccination, though recommended by Zammitt and Debono (1933), has so far not proved very successful. The ideal method is a policy of eradication (see p. 1715). All animals should be subjected to an agglutination and a skin test, positive reactors should be slaughtered, and breeding should be carried

out only from apparently uninfected animals. Herd tests should be made at 3-monthly intervals till no further reactors appear. If this policy is impracticable, the spread of infection should be limited as far as possible by elimination of all animals excreting the organism in the milk, by provision of separate boxes at the time of lambing or abortion, by abolition of communal pastures, by restriction of movement of animals from infected herds, and by control over the sale of animals for breeding. (For references to the general epizootiology and bacteriology of the disease see Report 1905–07, Segni 1931, Ananiadés and Miaoulis 1931, Dubois and Sollier 1932*b*, Pérès 1934, Moreno 1934, Meyer and Eddie 1935, Polding 1937, 1939, Taylor *et al.* 1938*b*, Zdrodowski 1938.)

It may be noted that abortion of sheep and goats is sometimes due to infection with *Salm. abortus-ovis*, a member of the *Salmonella* group (see Chapter 30).

CONTAGIOUS ABORTION IN CATTLE

Avortement épizoötique (French). *Seuchenhaftes Verwerfen* (German).

Contagious abortion in cattle is an infectious disease, due in the majority of cases to *Br. abortus* of Bang, and characterized by inflammatory changes of the uterine mucosa and fœtal membranes, resulting as a rule in the premature expulsion of the fœtus. Though regarded as contagious by Hutrel d'Arboval (1826) and Youatt (1834), (see Hutyra and Marek 1922), its infective nature was not shown definitely till 1878, when Lehnert transmitted the disease by intravaginal inoculation of pregnant cows with the vaginal discharge and placental tissue of aborting animals. In 1897 Bang, working with Stribolt, demonstrated microscopically a small Gram-negative bacillus in the uterine exudate of a cow with impending abortion, and succeeded in isolating it in pure culture. The intravaginal injection of this organism into two pregnant cows gave rise to abortion, and from the uterine exudate the organism was recovered in each case. Bang's work was confirmed by Preisz (1903) and Nowak (1908) on the Continent, by M'Fadyean and Stockman (1909) in this country, and by MacNeal and Kerr (1910) in America.

Epidemiology and Bacteriology

The disease is widespread throughout Europe, the United States of America, and most other countries of the world where there is a large cattle population. Its exact frequency is difficult to ascertain, but in this country Priestley (1934) estimates that 20 per cent. of the cows are infected. Similar high estimates have also been made for certain areas in the United States (Cotton 1931, Thomson 1932, Birch 1934). Large herds are more often infected than small. On account of the heavy loss from abortions, the frequent subsequent sterility of the animals, and the diminution in the milk yield, it is economically one of the most important diseases affecting cattle (Minett and Martin 1936). The fact, moreover, that infection may be transmitted to man renders the disease of public health interest.

Though clearly it is only pregnant animals that can display the typical symptom of abortion, infection may be conveyed by natural channels to cattle of any age and either sex. There is no distinct seasonal incidence. The commonest time for abortion to occur is from the 5th to the 8th month of pregnancy; 35·4 per cent. of cases occur in the 7th month alone (Wall 1911). Judged from experimental

work, the incubation period is variable; according to Mohler and Traum (1911) it may last from 1 to 33 weeks.

Introduced into a fresh herd the disease may spread rapidly, assuming epidemic proportions. Provided that no new animals are imported, it loses its initial severity, passing into an endemic state in which, if no preventive measures are taken, it remains for some years. A pregnant animal becoming infected for the first time generally aborts at an early stage; at its next pregnancy abortion occurs either not at all, or not till a later period; and though abortion may be repeated on a third occasion it is much commoner for the calf to be delivered at full term. Bang (1897) reports that of 83 cows only 30 aborted in 2 successive years, and only 6 three times in succession. From this it may be gathered that an immunity is acquired which is usually sufficient to protect the animal against further attacks of the disease.

The bacillus may, however, remain alive in the tissues of the cow for a considerable length of time. Bang (1897) found it in the uterus of a cow 9 months after the fœtus had died and become mummified. As a rule the uterus frees itself quickly, and the specific organism takes refuge in the udder and often in the supra mammary and pelvic lymphatic glands, where it may persist for months or even years (see Doyle 1935). Under favourable conditions it may be demonstrated in the blood stream, and occasionally in the urine and fæces (see Fitch, Bishop, and Boyd 1932*a*). Its frequency in the circulating blood is a little doubtful. Soule (1930) isolated it from about 10 per cent. of cows containing serum agglutinins, and Lübke (1935*a*), in a smaller series, from 22 per cent. Other workers, on the contrary, have failed to find it at all, or have found it in only a small proportion of animals (Fitch *et al.* 1932*a*, Krüger 1932, Doyle 1935, and numerous others). Probably the stage of the disease is important, and positive cultures are more likely in the early acute than the late chronic stage. Infection occurring in non-pregnant animals, particularly before sexual maturity is reached, may remain latent or may actually retrogress. In cows the organism frequently settles in the udder; abortion may or may not occur at the next pregnancy. In calves and young heifers the organism disappears completely from the tissues within a few weeks or months. In the udder *Br. abortus* gives rise to small subacute or chronic inflammatory foci situated in the alveoli, the interalveolar connective tissue, and along the lactiferous ducts (Runnels and Huddleson 1925, Ridala 1936). The lesions are too small to be detectable by clinical examination.

From the udder the organisms are excreted in the *milk*. This important fact was first demonstrated by Schroeder and Cotton in 1911. Karsten (1932) found that the proportion of aborting cows in which the milk was subsequently shown to be infected varied on different farms from 24 to 70 per cent., while in infected herds 19–33 per cent. of cows that did not abort excreted the organisms in their milk. Sheather (1923) observed that 34 per cent. of cows giving a positive blood serum agglutination reaction were yielding infected milk. Numerous similar observations have been made by different workers. For example, Pröscholdt (1932) found that 53 per cent. of cows with a blood serum agglutinin titre of 1/100 or over were excreting *Br. abortus* in the milk. The same author observed that, of 208 cows whose milk was infected, 145 had aborted, while 63 had calved normally. Lerche (1931) gives very similar figures. The organisms may appear in the milk within 1 or 2 days of abortion, and are usually demonstrable within a week, though in some animals as long as 5 months may elapse before excretion commences (Wall 1930). Many

cows cease excreting the organism after a few weeks, but others may remain infected for a year or two, or even permanently. In Pröscholdt's series, out of 145 aborting animals whose milk was infected, 22 had aborted 1–2 years previously. The excretion is irregular and often intermittent. The number of organisms present fluctuates from day to day. It is usually highest at the commencement of lactation, and gradually falls. Except in the first week or two of lactation, when there may be as many as 200,000 per ml., it is uncommon for the milk on withdrawal to contain more than 30,000 per ml. (Bang and Bendixen 1932*a*, Stockmayer 1936); the usual number is 10–2000 per ml. (Karsten 1932). Infection of the milk may be as heavy after normal calving as after abortion (Stockmayer 1936). Disease is often confined to one or two quarters of the udder. The hind-quarters are more often affected than the fore, and the right hind-quarter seems to be affected more often than the left (Bang and Bendixen 1931, Thompson 1934, Doyle 1935).

Though the organism commonly responsible for the disease is *Br. abortus*, numerous observations have shown that cattle in close contact with infected goats or sheep may become infected with *Br. melitensis* (see Taylor, Vidal, and Roman 1934, Benussi 1935). The infection seems to be localized mainly in the udder, and abortion is uncommon. Similarly in large hog-raising districts, cattle may become infected with *Br. suis* and excrete this organism in the milk (see Beattie and Rice, 1934, Molinelli and Ithurrat 1934).

Mode of Infection and Experimental Reproduction of the Disease.—There has been a long dispute over the commonest mode of infection. Cattle are so easy to infect experimentally, not only by dirt inoculation into the tissues, but also by the natural passages, that it is very difficult to decide on the route usually followed in the natural spread of disease. The three main portals are:

(1) The Mouth.—Bang (1906) and others have shown that it is possible to reproduce the disease in pregnant heifers and cows by feeding them with infective material, and have therefore suggested that infection probably occurs through contaminated fodder. It is known that the uterine exudate which is voided at the time of the abortion is extremely infective, that the bacilli may remain alive in it for some time—for 7 months if kept in the ice-chest (Bang 1897); that the aborted fœtus or fœtal membranes deposited on grass and left at atmospheric temperature shielded from direct sunlight may remain infected for as long as 6 months (Bosworth 1934–35); and that unless strict isolation of the animals is practised, there is ample opportunity for contamination of the pasture and other food material. A further source of infection is the milk of the aborting cows, which often contains *Br. abortus* in large numbers. Rats have been accused of spreading infection (Karkadinovsky 1936), but there is little evidence to support this (see Bosworth 1940).

(2) The Vagina.—In his original experiments Bang (1897) demonstrated the possibility of infecting pregnant cows by intravaginal inoculation of pure cultures. This has been confirmed frequently (Report 1909, M'Fadyean *et al.* 1913, Seddon 1919). It seems, therefore, not improbable that contamination of the vagina with infected soil or litter may be responsible for naturally acquired infection. A second method by which vaginal infection may occur is sexual intercourse. Bulls may be infected by injection of pure cultures under the prepuce (M'Fadyean *et al.* 1913). Injected intravenously, the organisms may lodge in the testicles and be excreted in the seminal fluid (Seddon 1919). Experimentally these facts are sufficient to indicate that the bull may play an active part in conveying the disease to cows. There is still stronger evidence from the epidemiological side, sufficient without doubt to incriminate the bull (Bang 1897). Moreover, the agglutination test shows that bulls are not infrequently infected by *Br. abortus*; Holth (1911) found agglutinins in the blood of 3 out of 28 bulls which he examined.

(3) THE SKIN AND CONJUNCTIVA.—Attention has been drawn to these routes of infection by Bang (1931), Bang and Bendixen (1932*b*), Cotton and Buck (1932), and Cotton, Buck and Smith (1933). Experimentally it has been found possible to infect animals fairly readily with *Br. abortus* by means of an infected compress left for a variable length of time in contact with the abraded, or even intact, skin. Infection has also followed simple conjunctival instillation of an *abortus* suspension.

Which of these three routes is the most important in practice, we have at present no exact means of telling. The Departmental Committee set up by the Board of Agriculture to investigate the disease considered that feeding was the most common method, that vaginal infection held second place, and that sexual infection probably played quite an insignificant part. Contamination of mucosal and skin surfaces must almost certainly be responsible at times for infection, but on the whole the prevalent opinion still seems to favour the alimentary route (see Mieszner 1931). Considering the frequency with which *Br. abortus* is excreted in the milk, and the ease with which animals can be infected by the udder (Schmidt 1932), it seems probable that infection must at times be transmitted from infected to normal cows at the actual time of milking. Unless the normal animals are milked first, contaminated milk must be rubbed into the teats, thus giving the organisms an opportunity to penetrate the skin or to enter the teat canal. There is still a wide field open for investigation. *The aim must be, not to ascertain whether a given route of infection is ever taken under natural conditions, but what the frequency distribution of the possible routes of infection really is.* Exact knowledge of this type may prove of great service in controlling the spread of infection in the field.

That the experimental reproduction of the disease can be readily accomplished by oral and parenteral inoculation was shown by the earlier workers. The interest of these experiments centres on the effect produced at different stages in the animal's life history. It is abundantly clear that inoculation during pregnancy is frequently followed by abortion, and by permanent infection of the udder and lymphatic system. The time between infection and abortion is said to be inversely proportional to the length of pregnancy (Thomsen 1937*b*). In calves and non-pregnant heifers, on the other hand, infection appears to be restricted to the reticulo-endothelial system (see Lübke 1935*b*), and usually dies out within a few weeks or months, leaving behind it an appreciable degree of immunity to fresh infection. This behaviour is now made use of in prophylactic vaccination (see p. 1716). The agglutinins that occur as a response to infection in the calf generally disappear within a year.

We may add that cattle may be infected, not only with *Br. abortus*, but also with *Br. melitensis* and *Br. suis*. These organisms settle readily in the udder, but less frequently give rise to abortion (see Zeller and Beller 1934, Thomsen 1934). Provided freshly isolated smooth strains are used, *Br. abortus* from the blood of human patients suffering from undulant fever seems to be fully virulent for young heifers (Birch and Gilman 1935). There is no satisfactory evidence that *abortus* strains of human and bovine origin differ in virulence for man or animals, though few direct observations, with the necessary precautions, have yet been made on this point.

Diagnosis

In the yellow or dark-brown mucoid exudate found between the uterine mucosa and the chorion, large numbers of *Br. abortus* may be demonstrated microscopically, aggregated into clumps within the polymorphonuclear cells. The organism may

likewise be seen in smears made from the gastro-intestinal contents of the fœtus. Provided the material sent for examination is fresh and uncontaminated, a provisional diagnosis may often be made microscopically ; as a rule, however, cultural or inoculation methods are required.

Cultivation.—The organism is best grown by seeding glycerine, or liver, agar slopes with the uterine exudate, or with the stomach contents of the fœtus, and incubating them in an atmosphere of air containing 5–10 per cent. of added CO_2 (Smith 1926, Huddleson *et al.* 1927). (See Chapter 34.) If uncontaminated, the organisms will form characteristic lenticular colonies in the course of 2 or 3 days (Fig. 173, p. 819). Their identity should be confirmed by agglutination. The bacilli are sometimes found in the blood and in the liver of the fœtus, but less abundantly than in the stomach. If the material is not pure, it is best to resort immediately to guinea-pig inoculation. The material is ground up in Ringer's solution, and 0·5 ml. injected intramuscularly into two or three guinea-pigs. The animals are killed after 4–8 weeks, the blood is examined for agglutinins, and cultures are made from the sublumbar glands and the spleen (see "Milk" below). At post-mortem examination of slaughtered cattle the organisms are most likely to be found in the udder, and in the supramammary, iliac, and pharyngeal glands. They may be sought for by direct culture and by guinea-pig inoculation (see Doyle 1935). Any obvious lesions, such as hygroma of the knee (v. d. Hoeden 1932*a*), should also be examined.

Agglutination Reaction.—In practice this is the most widely used method of diagnosis. Although the agglutination test in cattle does not differ in principle from that in man, it is necessary to consider separately its interpretation in relation to the titre that may be observed.

The test may be carried out by the tube or the slide method (for technique, and preparation of antigens, see Huddleson and Abell 1928, Huddleson 1932, 1939, Welch and Mickle 1933, Fitch and Donham 1933, Donham and Fitch 1934*a*, *b*, 1935, Welch and Marsh 1935). Final serum dilutions of 1/10–1/2560 should be put up. Intermediate zone phenomena are frequently encountered (see Priestley 1931).

The interpretation of this test is often difficult, and is not rendered any easier by the fact that, owing to differences in technique, the titres of different workers may not be strictly comparable.

Calves, even those born of infected mothers, give a negative serum reaction at birth. If, however, they are allowed to suck infected dams within the first 24 hours of life, agglutinins become demonstrable in their serum within about 2 hours. This appears to be due to the absorption through the alimentary tract of antibodies present in the colostrum. Calves passively immunized in this way lose their agglutinins very rapidly (McAlpine and Rettger 1925). Even calves that are actively infected and develop agglutinins some time after birth appear to lose them within 6 months, unless reinfection occurs (Thorp and Graham 1933).

In an animal that is infected during pregnancy the titre generally rises before abortion to 1/200–1/1000 or over. During the following 6 months it tends to fall. If the cow becomes a chronic carrier, the titre usually remains fairly high. About 80 per cent. of cows with a persistent titre of 1/200 or over are found to be excreting *Br. abortus* in the milk, while a titre of 1/1000 or over is almost diagnostic of udder infection. If animals are followed for any considerable length of time, the agglutinin titre of positive reactors will often be found to decline. This is particularly true of low reactors. Damon (1932), for example, who observed a herd with a mean complement of 225–250 animals over a period of 4 years, found that 27·7 per cent. of animals reacting at 1/25, 17 per cent. of those reacting at 1/50, and 5 per cent. of those reacting at 1/100 or over lost their

agglutinins permanently (see also Hadley and Welsh 1931). Huddleson and Smith (1931), however, found that, of a total of 247 animals reacting at 1/25 or over, and followed up for a period of 1–8 years, only 4 became permanently negative. Animals that abort usually have a considerably higher titre than non-aborting animals. A few animals may fail to show any agglutinins at the time of abortion, and for a few days afterwards; most of these become positive within 2–3 weeks, but there is some evidence that others may fail completely to develop agglutinins. Animals may certainly excrete *Br. abortus* in the milk and yet fail to show agglutinins in the blood serum or whey (Karsten 1932).

The interpretation of a single test is often difficult, and depends on whether infection is known to be present in the herd. Many workers regard a titre of 1/20 or 1/25 as strongly suggestive of infection, whereas others pay little or no attention to a titre of less than 1/100. Our own view is that a titre of 1/25 cannot be disregarded, particularly if occurring in an infected herd. The only satisfactory method, however, is to make monthly tests on all suspicious animals, so as to detect as soon as possible those that become definitely positive. It must always be remembered that, though a positive test is a practically certain indication of infection, a doubtful or negative test cannot be held to exclude infection. (For interpretation of reaction see also Zwick 1910, Grinstadt 1910, Wall 1911, M'Fadyean and Stockman 1912, Seddon 1919, Smillie, Little and Florence 1919, Mathews 1924, Jordan and McBroom 1932, White *et al.* 1933, Ranney 1936, Kitselman 1936, Fitch *et al.* 1937.)

Complement-Fixation Reaction.—The complement-fixation test gives results very similar to those of the agglutination test. Mohler and Traum (1911), who examined 400 sera by both methods, found close agreement between them; in only five instances were the results at variance. Similar results were recorded by Wall (1911), Reinhardt and Gauss (1915), Zwick (1910), and others. Mitchell (1929) considers that both tests are desirable, while Lentz (1932) prefers the complement-fixation test. Holth (1911) demonstrated the value of the complement-fixation test in cases of abortion in which the products obtainable for examination were badly contaminated; if the placenta is ground up in saline, heated to boiling-point and filtered, it can be used as an antigen.

The Abortin Reaction.—Abortin is a substance analogous to tuberculin, which was introduced by M'Fadyean and Stockman (1909) in an attempt to elaborate a diagnostic test based on the hypersensitiveness of infected animals. It is prepared from a 4 to 6 weeks' glucose glycerine broth culture, which is sterilized at 99° C. for 2 hours, filtered through a Berkefeld candle, evaporated to 1/10 of its bulk, and preserved with 0·5 per cent. phenol. The dark syrupy liquid thus obtained is suitably diluted before use, and injected subcutaneously. In a positive reaction the temperature rises to 104–106° F. As a method of diagnosis, this original abortin did not find favour; the authors themselves after extensive trials were disappointed with it (Stockman 1914). Nor did Holtum's (1928) heat-killed suspension of *abortus* in phenolized saline meet with success. Probably a bacillary extract, such as that prepared by Huddleson and Johnson (1933) or Taylor, Lisbonne, and Vidal (1935) might be found satisfactory. Further observations are badly needed on the use of this test, preferably carried out by the intracutaneous inoculation of abortin into the caudal fold. A few promising reports of the test have been made of recent years (Dubois and Brune 1933, Dubois 1934, Rossi and Vigel 1934, Franco and Pezzi 1935), and it might prove valuable in the detection of agglutinin-negative animals. Mirri (1935) recommends a palpebral test.

Demonstration of Br. abortus in Milk.—Examination for the presence of *Br. abortus* in milk may be conducted by cultural or by animal inoculation methods.

Cultivation.—Cultural methods are satisfactory only with milk samples from individual cows collected under relatively aseptic conditions. Separate quarter samples should be taken, and either kept refrigerated, or preserved with 0·5–1·0 per cent. boric acid, till the time of examination. The whole milk, the gravity cream, or the deposit from high-speed centrifugation should be streaked on to a number of plates of liver extract agar, or 2 per cent. glycerol agar containing 10–15 per cent. of ox serum. Contaminating organisms may be partly suppressed by the addition to the medium of gentian violet and malachite green. The exact concentrations of these dyes vary according to the manufacturer; usually a final concentration of 1/100,000 to 1/200,000 is satisfactory. The plates should be incubated in 5–10 per cent. CO_2, and examined at intervals for 14 days (see Huddleson *et al.* 1927, Hasley 1930, Traum and Henry 1930, Karsten 1932, Pröscholdt 1932, Karsten and Bischoff 1933, 1934, Stockmayer 1933*a*, 1935).

Animal Inoculation.—If the animal method is chosen, it is advisable to inoculate a mixture, composed of 2 ml. of gravity cream and the deposit from 100 ml. of milk after high-speed centrifugation, intramuscularly into the hind leg of each of two guinea-pigs. Alternatively 4 ml. of whole milk may be injected, 2 ml. into each thigh. The animals should be killed about 6 weeks later. At post-mortem examination the femoral and sublumbar glands will be enlarged and pale; the spleen may be enlarged, its surface slightly irregular, and a few small greyish-yellow necrotic foci may be present. The liver may show two or three tiny necrotic foci. The macroscopic lesions are often inconspicuous, and must on no account be relied upon for diagnosis. The blood serum should be tested for agglutinins; a titre of 1/25 or over is highly suggestive of infection. Cultures should be made from the sublumbar glands and spleen, and all suspicious organisms identified by agglutination and other methods. In not all animals containing serum agglutinins is it possible to isolate the organisms from the tissues. On the other hand, it is uncommon to isolate them in the absence of a positive agglutination reaction. Individual guinea-pigs vary considerably in their susceptibility to *Br. abortus*, and it is common to obtain positive results in one animal and negative in the other (see Smith 1932*b*, Plate 1934*a*). If tubercle bacilli are present in the milk simultaneously with *Br. abortus*, the guinea-pig will suffer from a double infection. The diagnosis of tuberculosis can be made on the basis of the macroscopic lesions and the demonstration of acid-fast bacilli in the organs. The diagnosis of *abortus* infection can be made on the basis of the agglutination reaction and the cultivation of the organisms from the tissues. Pullinger (1936), however, has pointed out that the tubercle bacillus interferes with the development of *Br. abortus* in the animal body, so that the demonstration of this organism may often be unsuccessful in milk coming from cows with tuberculous mastitis.

All workers are agreed that the animal inoculation method is more satisfactory for the demonstration of *Br. abortus* in milk than the direct cultural method. Plate (1934*b*) found that 90 per cent. of infected samples were positive by the animal and only 50 per cent. by the cultural method. Similarly Karsten and Bischoff (1934), who examined 466 milks by both methods, obtained 184 positive results by the guinea-pig and 101 by the cultural method. It is not uncommon to obtain occasional positive results by culture, when the animal inoculation results are negative, so that it is advisable to use both methods. In mixed milk, or in milk of single animals that has not been drawn aseptically and kept cold, the animal inoculation method is the only suitable means of demonstrating *Br. abortus*.

Serological Examination of Milk.—Indirect evidence of infection of the milk may be obtained by examination of the whey for agglutinins or complement-fixing bodies. The whey may be obtained by adding 1 drop of rennet to 5 ml. of skim milk, incubating at 58° C. for 30 minutes, breaking up the clot, and centrifuging. Alternatively 5 ml. of carbon tetrachloride may be added to 10 ml. of milk, together with a small quantity of rennet. The milk is corked, and shaken for several minutes till the fat is extracted.

It is then incubated for 1 hour, and centrifuged (Hall and Learmonth 1933). The advantage of 58° C. for incubation is that it destroys any complement present. The agglutination test is carried out in the usual way. Control tests must be put up, since non-specific reactions may occur. A whey agglutinin titre of 1/80 or over affords a high probability that the udder is infected and that *Br. abortus* is being excreted in the milk. The higher the titre, the more probable this is. Similarly, fixation of complement with 0·05 ml. of whey is strongly suggestive of milk infection.

Examination of the whey for antibodies is sometimes used as a method of detecting infection of the animal with *Br. abortus*. Except as a preliminary means of investigation, this procedure is unsatisfactory, because only a proportion of infected animals containing blood serum agglutinins give a positive whey reaction. (For references to use and interpretation of whey test, see M'Fadyean and Stockman 1909, Seddon 1915, Robinson 1919, Sheather 1923, Graham and Thorp 1930, Gilman 1930, Hasley 1931, Norton and Pless 1931, Berge and Ekrem 1932, 1934, Pröscholdt 1932, Karsten 1932, Karsten and Bischoff 1933, 1934, 1935, Plate 1934*a*, *b*, Caldwell, Parker, and Medlar 1934, Molinelli and Ithurrat 1934, 1935, Smith 1934*a*, *b*, van Oyen 1934, Doyle and Beckett 1936.)

Prophylaxis and Treatment

At the time of abortion the uterine discharge is highly infectious; the usual measures must therefore be taken for isolation of the animal, and subsequent disinfection of its stall. The fœtus and membranes should be burned, or buried in lime. Irrigation of the cow's vagina should be practised with an antiseptic solution for a week or till all discharge has ceased, and not till some considerable time has elapsed since the complete cessation of the discharge should the cow be taken to the bull. If a bull has served an animal that has recently aborted, it is well to irrigate its penis and preputial sheath with an antiseptic solution, in order to prevent active infection of the bull, or passive carriage of the organism to another cow. Calves born of infected cows are better brought up on milk from another animal.

Eradication of Contagious Abortion.—The ideal policy is the complete eradication of infection. Various methods are recommended for doing this. The general principle consists in the detection of infected animals by the agglutination or complement-fixation reaction, the segregation or sale of positive reactors, and the building-up of a clean, non-infected herd. The degree of success attending this policy varies with a number of factors. With a small or medium-sized herd, particularly if self-contained and protected against infection from water, manure, and other animals, with provision of calving boxes, and re-testing of non-reactors every 2 months so as to eliminate all animals as soon as they become positive, it is often possible to eradicate the infection entirely and to maintain a healthy herd for several years. On the other hand, with very large herds, particularly if not self-contained, with flying herds, with farms on which adequate accommodation for segregation of infected, and quarantine of newly imported, animals is impossible, and with imperfect control over infection from other sources, the results are often disappointing. Generally speaking, if the conditions are favourable, and if the disease is not at the height of its activity, this policy should be adopted, since its success is followed by the improved general health of the herd, better and more regular breeding, and an increased milk supply. Success, however, cannot be guaranteed. One of the greatest problems at the moment is presented by the infected non-reactor, which may abort, or excrete *Br. abortus* in the milk, and so contaminate the other animals in the herd (see Bang 1906, M'Fadyean 1921, 1924, Giltner 1924, Thomsen

1928, Zeller 1931, Henricsson 1932, Kitselman 1932, Newsom and Cross 1932, Clark 1932, Mitchell *et al.* 1933, Fritz and Barnes 1933, Birch 1934, Birch *et al.* 1934, van Oyen 1934, Report 1935, Thornshaug 1937, Gilbert 1937).

Vaccination.—Early observations by Bang (1906), M'Fadyean and Stockman (1909) and Stockman (1914) suggested that the inoculation of non-pregnant heifers or cows with a living vaccine of *Br. abortus* was able to confer some degree of immunity on the animals, as shown by a lower abortion rate. During the next 20 years or so vaccination, usually with strains of modified virulence, was used fairly extensively, and numerous papers were published on the results (for references, see Topley and Wilson, 2nd ed., p. 1355). Little fresh knowledge, however, was gained, chiefly because few observations with properly controlled groups of animals were made. More recently a great deal of attention has been devoted to the preparation of a standardized vaccine, and much valuable information has been forthcoming. It has been found that the more virulent the vaccinal strain is, the greater is the degree of subsequent immunity; but that in practice the use of a fully virulent strain is inadvisable, because of the danger partly to pregnant animals in the herd and partly to the human population if it is excreted in the milk. A strain of modified, and preferably fixed, virulence is preferable. Such a strain was introduced by Buck (1930) in the United States under the name S 19, and has since been used with good effect for the vaccination of calves and non-pregnant heifers and cows (for references, see Haring 1938, 1939, Haring and Traum 1937, 1941, McEwen 1941, Huddleson 1942). There is some evidence that the immunity so conferred persists even to the second pregnancy, but it is probably wiser to revaccinate the animals before being taken to the bull. One disadvantage of vaccination with the S 19 strain is that it stimulates the formation of antibodies and thus interferes with the normal interpretation of the agglutination reaction. To overcome this objection, McEwen (1940*b*) has introduced another strain of modified virulence, Strain 45 (20), which does not call forth agglutinins and which can therefore be used in clean herds in which the eradication policy is being pursued.

It is still too early to say how far the use of live vaccines will succeed in paving the way towards the complete elimination of contagious abortion. Present evidence suggests that it raises the degree of immunity of the individual animal to a level at which multiplication of the fully virulent organism, when this gains access to the tissues by natural channels, is restricted. Unlimited multiplication in the uterus and udder is prevented, so that abortion and infection of the milk are much less frequent than in uninoculated animals. This, in its turn, should lead to a diminution in the total amount of infective material excreted, and thus lessen the danger of infection both of other animals in the herd and of human beings consuming raw milk.

One danger of the live vaccine is that the strain may regain its virulence in the animal body. That this is not a hypothetical risk has been shown by McEwen (1940*a*). For this reason attempts are still being made to make use of a *dead vaccine*. In spite of the earlier statements, there seems no reason why properly killed organisms, given with due regard to size and spacing of dosage, should not afford protection almost as good as that conferred by living organisms. Already Huddleson (1943) has obtained promising results on guinea-pigs with organisms killed by mechanical crushing, and experiments in this country with organisms killed in a different way have yielded very hopeful results.

BRUCELLA INFECTION OF SWINE

This disease has only recently been recognized, and is of limited distribution. It first attracted serious attention in the large hog-raising districts of the middle western States of North America. According to the observations of Boak and Carpenter (1930), Feldman and Olson (1934), and McNutt (1935) about 2 per cent. of animals destined for the abattoirs are infected in this area. In Europe, the disease has been recognized in several countries, but with a few exceptions it appears to be mainly of sporadic occurrence (Thomsen 1931, 1934, 1937*a*, Knoth 1930*b*, Saxer 1936, Steffens 1937). A focus of infection has been found in White Russia (Makkawejski *et al.* 1933). The disease appears to be not uncommon in Brazil (see Neiva 1934) and the Argentine. It has been observed in New South Wales (King 1934). In this country it has not so far been detected (see Doyle 1934).

Bacteriologically, all outbreaks appear to have been due to *Br. suis*, and all of them, with the exception of the Danish epizoötic, to the American type (see Chapter 34), though complete information has not always been available on this point. The disease is essentially an infection of the reticulo-endothelial system, with the production of inflammatory disturbances in the glands, joints, and reproductive organs. Contrary to contagious abortion of cattle, in which the females are chiefly affected, the disease in swine attacks mainly the *boars*. Abortion, though it does occur, is much less frequent than in cattle. Abscess formation is met with in the submaxillary, cervical, inguinal, and popliteal lymph glands. The joints of the hind legs, particularly the knee and hock, are frequently affected; they are swollen, painful, frequently contain fluid, and sometimes ankylose. Epididymo-orchitis is very common. In Makkawejski's series, no fewer than 269 out of 786 boars showed orchitis, usually bilateral. The affected testicles are sometimes of enormous size, reaching 6½ lb. in weight, and purulent foci, which may undergo calcification, are not infrequent. Multiple foci may also be present in the vesiculæ seminales. *Br. suis* may, however, often be demonstrated in the tissues in the absence of macroscopic pathological changes.

In *sows* a condition for which Thomsen (1934) suggests the name "miliary brucellosis of the uterus" is frequently met with. The interior of the uterus is studded with small yellowish-white nodules located in the deeper layer of the mucosa and projecting slightly above the surface.

The disease can be readily reproduced by feeding and by conjunctival inoculation. According to Stockmayer (1933*b*), pigs are susceptible to *Br. abortus* as well as to *Br. suis*, though no outbreaks due to *Br. abortus* have yet been recorded in the field. Thomsen believes that natural infection is spread to a considerable extent by copulation, though this of course will account only for transmission of the disease to sexually mature animals. Infection of the conjunctiva by urine, and alimentary infection probably play a part. Sucking pigs are readily infected. It is possible that infection occurs through the milk of the mother, though invasion of the udder is uncommon.

In the field, *diagnosis* is best made by the agglutination and complement-fixation tests on the blood serum. A titre of 1/100 is regarded as definitely positive, 1/50 as strongly suggestive, and 1/25 as doubtful. Some help may be given by the intradermal test (Thomsen 1934). In the abattoir, the blood serum should be examined, and a search made by culture and guinea-pig inoculation for *Br. suis*

in the tissues. According to Johnson and Huddleson (1931) and Johnson, Huddleson, and Hamann (1933), the organisms are commonest in the spleen, gastric and supramammary lymph glands, and the liver. Numerous other organs may be infected, but less frequently.

The disease is of undoubted economic importance, owing particularly to impotence in the boars and abortion or sterility in the sows. But it is probably of even more importance in its effect on public health. Already large numbers of cases of undulant fever have been ascribed to it, and it affords a very real hazard for workers in abattoirs and packing houses. So far as reports go, it would seem to be fairly amenable to control by the eradication policy. In Denmark, by the institution of such a policy, involving slaughter of infected animals in lightly infected herds, and slaughter of all animals in heavily infected herds, it proved possible to stamp out the disease entirely within a very short time. Johnson, Huddleson, and Hamann (1933) in the United States have been successful with a less radical policy, involving blood testing once a month, followed by segregation and ultimate elimination of the positive reactors. The seasonal breeding of sows makes control of the disease considerably easier than in cattle. (For further information on this disease the reader is referred to the excellent monograph of Thomsen 1934.)

BRUCELLA INFECTION OF HORSES, DOGS, CATS, FOWLS AND RATS

Horses.—Following on the work of Rinjard and Hilger (1928) in France, *Brucella* infection of horses has now been recognized in a number of European countries (see van der Hoeden 1931, Makkawejsky *et al.* 1931, Panisset and Delbé 1932, Hieronymi and Gilde 1934, Duff 1937, Thomsen 1937*a*), in the United States (Fitch, Delez, and Boyd 1930), and in Australia. In Holland van der Hoeden (1932*d*) found that 72 out of 482 horse sera, mostly taken from the slaughter-house, agglutinated *Br. abortus* to 1/100 or over. In this country Priestley (1934) obtained evidence that 5–15 per cent. of horses had specific antibodies in their blood. The incidence in males and females was much the same, but older horses seemed to be more often infected than younger. Since agglutinins are said to be present in normal horses up to a titre of 1/50 or 1/100, estimates of the incidence of infection based on agglutinins alone should be accepted with caution (see Krüger 1937, Deem 1937). The disease may be contracted from other horses or from cattle. Though the infection frequently remains latent, in a certain proportion of animals it is accompanied by the appearance of suppurative lesions, particularly on the head and neck (poll-evil, fistulous withers), and less often of bone abscesses, arthritis, and tenosynovitis. Their appearance may be preceded by fever and weakness. No history of local trauma is usually obtainable. The inflammation is extremely painful, and lasts for a few days to several months. The abscess may disappear by absorption, but more often it breaks down at multiple points on the surface, and discharges a yellowish viscous fluid containing fibrinous clots. Bacteriologically *Br. abortus* can usually be isolated, or sometimes in the United States *Br. suis*, though secondary infection of the fistulæ readily occurs. Horses can be infected experimentally with *Br. abortus* by feeding or parenteral inoculation (v.d. Hoeden 1932*b*, *d*, Schneller 1934); but according to Fitch, Bishop, and Boyd (1932*b*) the local manifestations of the disease cannot be reproduced except by direct inoculation

into the neck ligament. The opinion is expressed by van der Hoeden (1932*b*, *d*) that poll-evil is essentially an allergic manifestation. It was found, for example, that if dead *abortus* bacilli were injected subcutaneously into an infected animal, a local abscess formed from which living organisms could be cultivated. Bacteriological diagnosis of infection is made by the serum-agglutinin test ; a titre of 1/200 may be regarded as positive, and 1/100 as suspicious. An allergic test may also be used, the ophthalmic (v.d. Hoeden 1932*b*, *d*) or the intradermal (Rossi and Saunié 1934) route being chosen. Horses may convey infection to cattle (White and Swett 1935), and in any eradication scheme the two animals should be kept separate.

Dogs.—*Brucella* infection of dogs appears usually to be of the inapparent type. Symptoms of disease are uncommon, and though occasionally metritis or local abscess formation may occur, the infection can usually be diagnosed only by examination of the blood serum for agglutinins and complement-fixing bodies. The disease seems to be not infrequent in both Europe and America. In some surveys 2–10 per cent., or even more, of dogs have been found to be infected (v.d. Hoeden 1932*c*, Thomsen 1932*b*, Grandi 1933, Feldman, Mann, and Olson 1935), though caution must be observed in drawing conclusions on the frequency of infection from serological results alone. The disease can be reproduced by feeding and by parenteral inoculation, and may be characterized by fever of an undulating type. In both naturally and experimentally infected animals the organism may sometimes be demonstrated in the blood stream or urine (v.d. Hoeden 1932*c*, Thomsen 1932*b*, Feldman, Bollman, and Olson 1935, Olson and Feldman 1936). Most cases appear to be due to *Br. abortus*, but at least one *suis* infection has been described (Planz and Huddleson 1931). Though dogs probably play little part in the general spread of *Brucella* infection, their possible rôle as carriers must be considered in attempts to eradicate the disease from cattle or other animals.

Little is known about the incidence of *Brucella* infection in *cats*, though considering their habit of drinking raw cows' milk it is difficult to believe that they are not frequently infected. Experimentally, both *Br. abortus* and *Br. melitensis* may give rise to a severe disease with lesions in the joints and internal organs (Makkawejsky and Karkadinowskaja 1932).

Fowls.—The frequency of *Brucella* infection in fowls is still under discussion. Emmel and Huddleson (1929, 1930) brought evidence to suggest that the disease was very common in the United States, but their observations have not been altogether confirmed by subsequent workers (McNutt and Purwin 1930, v. Roekel *et al.* 1932). It is noteworthy that their conclusions were based on the presence of agglutinins in the blood. The isolation of the organisms from the tissues of naturally infected birds seems to have been very rarely accomplished. Beller and Stockmayer (1933*a*) have shown that normal fowls frequently have agglutinins in their blood, and that no attention can be paid to a titre of less than 1/150 or 1/200. Most workers have found that experimental inoculation of fowls gives rise to an inapparent infection, having little or no effect on the health or egg-laying capacity of the birds (see McNutt and Purwin 1930, 1932, v. Roekel *et al.* 1932, Lombardo 1932, Beller and Stockmayer 1933*a*, *b*, and Chapter 34). The natural infection seems to be more of epidemiological than clinical interest, though further observations are required, particularly to find out whether fowls may transmit infection to cattle.

Rats.—Little is known of the occurrence of natural infection in wild rodents, though, in view of the possible part played by these animals in the spread of infection among cattle, knowledge is urgently required. Karkadinovsky (1936) examined 34 wild grey rats on three farms infected with contagious abortion, and isolated *Br. abortus* from 11 of them. Most of the positive cultures were from the spleen and liver, but in two of the animals the blood was also found to be infected. Bosworth (1940), on the other hand, who examined 167 rats caught on farms where the disease was prevalent, found only one harbouring *Br. abortus*. The observations of Bosworth (1937) and of Sandholm (1938) show that rats can be infected by feeding, and that the organisms can be excreted for a short time in the faeces and urine, but that on the whole the animals are relatively resistant and that tissue lesions seldom occur.

Infectious Abortion in Cattle and Sheep due to *Vibrio fetus*

In 1913 M'Fadyean and Stockman (Report 1913) described an enzootic abortion of sheep due to a spirillar organism, which was later called *Vibrio fetus* by Smith (1918). In this country, vibrionic abortion appears mainly to affect sheep, though the disease has been observed in cattle; but in the United States it is responsible for a considerable proportion of cases in cattle. Smith (1919) found, for example, in 109 cases of abortion in which a relatively thorough examination of the fœtus and membranes was made, that 62 were due to *Br. abortus*, 26 to *V. fetus*, 2 to *C. pyogenes*, while in the remaining 19 no organisms were isolated or mixed cultures were obtained. The general epidemiology and bacteriology of vibrionic abortion are similar to those already described for the more common type of abortion. Thus infection appears to occur by feeding, and possibly by vaginal contamination: the vibrios are found in large numbers in the uterine exudate, in the placenta, and in the contents of the fœtal stomach: agglutinins are present in the serum of infected animals, active up to a dilution of 1/610 or even higher (Smith *et al.* 1920): and pregnant cows can be infected by the mouth, though more certainly by intravenous inoculation.

An organism similar in many respects to *V. fetus*, and called *V. jejuni*, appears to be responsible for *autumn diarrhœa in cattle* in the United States (see Jones and Little 1931, Jones, Orcutt, and Little 1931, and Chapter 22).

TULARÆMIA

Synonyms: Deer-fly fever, Pahvant Valley fever, Ohara's disease.

In 1911 McCoy described a plague-like disease amongst the ground-squirrels (*Citellus beecheyi* Richardson) of California. The following year McCoy and Chapin (1912) isolated the causative organism—a very tiny Gram-negative bacillus—from naturally infected animals, and reproduced the disease with pure cultures in guinea-pigs. They named it *Bact. tularense*—from Tulare, the county in which the disease was first observed. (This organism probably belongs to the *Brucella* group, see Chapter 34.) In 1921 the disease caused by this organism came to be known as tularæmia. It is apparently widespread amongst rodents in America, particularly in the Western States; ground-squirrels, hares and jack-

rabbits are the animals chiefly infected. The lesions found in animals dead of the disease are similar to those of plague; there is a bubo, generally in the cervical, axillary, or inguinal region, containing dry, yellowish, caseous material; the spleen is greatly enlarged, very dark in colour, and contains yellowish-white, discrete, caseous granules up to 1 mm. in diameter, projecting slightly above the surface; there are numerous granules in the liver; the lungs are rarely affected; the organisms are present in enormous numbers in the spleen, in smaller numbers in the liver, bubo, and heart's blood (McCoy and Chapin 1912). Experimentally the disease can be reproduced in ground-squirrels, gophers, guinea-pigs, rabbits, mice, and monkeys; rats are more resistant; cats, dogs, and pigeons appear to be immune. Feeding, nasal instillation, cutaneous, subcutaneous, intraperitoneal, and conjunctival injection are all successful (see Chapter 34.) The disease appears to be spread by blood-sucking insects, especially ticks (McCoy and Chapin 1912, Francis 1921, Francis and Lake 1922, Parker *et al.* 1924, 1929). According to Parker and Spencer (1926*b*) the organism may be transmitted from infected female ticks to their progeny; if this is true, it is one of the few examples known of the hereditary transmission of a bacterium by insects.

The first case of the disease in man was reported in 1914 by Wherry and Lamb in the United States. From 1924 to 1937 inclusive, 8,022 cases of infection with 358 deaths were recognized (Olson 1938), though there is reason to believe that numerous other cases occurred that were not diagnosed (see Francis 1921, Culpepper 1926, Dieter 1926, Freese *et al.* 1926, Lavan 1926, Maclachlan *et al.* 1926, Parker and Francis 1926, Cumming 1930). The disease has been met with in Japan, where it is known as Ohara's disease (see Ohara 1930), in Norway (see Wefring 1930), in Soviet Russia (see Doubrowinsky 1930, Karpoff and Antonoff 1936), in Sweden (Olin 1938*a*, *b*), in Austria and Czechoslovakia (Drbohlav 1937), and in Turkey (Arar 1937). Cases are mainly sporadic, but occasional outbreaks have been reported. The case mortality is about 5 per cent.

Mode of Infection.—Infection of man is most commonly due to the handling of infected rodents, and occurs either directly through contamination of the hands with the tissues or indirectly through the bites of parasites. In the United States about 90 per cent. of cases are traced to contact with wild rabbits and hares (Cumming 1937), but in some instances infection is transmitted by wood ticks (*Dermacentor andersoni* and *D. variabilis*) and deer flies (*Chrysops discalis*) (see Francis 1921, Parker and Francis 1926, Hillman and Morgan 1937, Olson 1938).

In Norway and Sweden infection has been traced to hares (Thjøtta 1930, 1931*b*, Olin 1938*b*), and in Soviet Russia to water rats (Sarchi 1929, 1930), and to susliks (Tumansky and Kolesnikova 1935). The "lemming fever" of Norway, which follows the consumption of drinking water polluted by the bodies and excreta of the lemming, is thought to be related to tularæmia (Thjøtta 1931*a*). Some cases in man have been traced to contact with sheep, which may occasionally suffer from tularæmia (Parker and Dade 1929, Philip and Jellison 1935). Case-to-case infection is said to be rare. The incidence of the disease is mainly on those classes of the population who are brought into contact with infected animals, such as butchers, poultry-men, and trappers. Laboratory workers are often attacked (Parker and Spencer 1926*a*). Indeed there is probably no other organism that is so dangerous to work with in the laboratory. Unless scrupulous care is taken to avoid infection of the skin, nose, and conjunctiva, from fluid cultures and inoculated animals, the disease will almost certainly be contracted.

The way in which the organism enters the body is not known, but the evidence suggests that the site of infection is generally the skin of the fingers or the conjunctiva. It is known that in guinea-pigs the organism can pass through the intact skin (Lake and Francis 1922), and there is reason to believe that it may do so in human beings, though infection is probably aided by the presence of small wounds and abrasions. The incubation period of the disease is generally stated to be about 3 days. In the usual glandular type of the disease, seen in butchers and poultry-men, after an acute onset with headache, rigors, pains and fever, a papule appears, generally on the back of the finger, which breaks down and leaves a ragged ulcer. The epitrochlear and axillary glands swell and become painful; they often break down and discharge purulent material. Fever is common for the first 2 or 3 weeks. Convalescence is very slow. When the organism gains entrance through the conjunctiva, ulcers may form on the inner surface of the eyelids, followed by swelling and tenderness of the preauricular and cervical glands. In the typhoid type of the disease there are no localizing symptoms. It is this type that is usually seen in laboratory workers (Lake and Francis 1922). Death, when it occurs, is commonest during the 3rd week. In those patients who have been examined at post mortem, multiple small abscesses in the regional lymphatic glands, the spleen, liver, and lungs have often been found (see Francis and Callender 1927, Foulger *et al.* 1932).

Diagnosis, Prevention and Treatment.—Diagnosis in animals is best made by macroscopic, microscopic, cultural, and pathogenicity tests. In the differential diagnosis from plague, account should be taken of the absence of pus at the site of infection, the greater variability in size of the granules in the spleen, the rarity with which the lungs are affected, and the failure of the organism to develop on ordinary media. In man, discharge from the local lesions or glands should be cultivated directly on to special media (see Shaw and Hunnicutt 1930), and injected into guinea-pigs (Francis 1923). The agglutination test is of special value (Francis and Evans 1926). It becomes positive, as a rule, during the 2nd week of the disease, and reaches its maximum between the 4th and 8th weeks. The titre varies from about 1/160 to 1/5,000. Sera agglutinating *Br. tularensis* at 1/320 or higher often act on *Br. abortus* as well. In some cases the two organisms may be agglutinated to the same degree. Agglutinins to *Br. tularensis* persist in low titre for years after the attack (Ransmeier and Ewing 1941). Curiously enough the organism does not often appear to be present in the blood (Lake and Francis 1922). Intradermal diagnostic tests have been described (Foshay 1932, 1936).

In animal experiments no cross-reactions are said to occur between "protein nucleate" solutions prepared from *Br. tularensis* and from the other three members of the *Brucella* group (Pennell and Huddleson 1941).

Prevention of the disease lies in avoiding contact of the skin with potentially infected material, such as wild rabbits and hares. Immunity after an attack is lasting. Persons exposed to risk of infection may be vaccinated prophylactically. Foshay and his colleagues (1942) recommend three doses of a vaccine made from organisms killed with nitrous acid, followed by annual revaccination. Immune serum prepared by the inoculation of a goat has been tried on a small scale for the treatment of patients suffering from the disease (Foshay 1934).

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CHAPTER 76

ANTHRAX

History

ANTHRAX is a disease that has been known from antiquity. In earlier days, however, it was not clearly separated from other affections closely simulating it. Maret (1752) and Fournier (1769) defined the clinical type of malignant pustule in man, and Chabert (1780) gave a clear description of anthrax in animals. In 1823 Barthelémy showed that it was transmissible by inoculation. Rayer (1850) described small, non-motile, filiform bodies in the blood of sheep dead of the disease, and confirmed its transmissibility by inoculation. (For references see Hutyra and Marek 1922.) In a series of papers, Davaine (1863*a*, *b*, *c*, 1864) showed that anthrax could be transmitted to sheep, horses, cattle, guinea-pigs, and mice, by the subcutaneous inoculation of infected but not of normal blood; that in such animals the bacilli did not appear in the blood till 4 or 5 hours before death; that in the blood they increased rapidly in numbers, and became filamentous; and that after death they disappeared as soon as putrefaction commenced. He showed, moreover, that the blood of an infected animal, previous to its invasion with the bacilli, was non-infective, but that after invasion it was capable of conveying the disease; that animals fed on infected viscera frequently became infected, but that animals fed on the putrefying organs of non-infected animals did not do so; and that after death from anthrax the spleen, liver, kidneys, lungs, blood, and, to a less extent, other organs contained the bacilli in large numbers. In the same year, Tiegel and Klebs (see Koch 1881) showed that anthrax blood, if filtered through a clay candle, was deprived of its infectivity; the filtrate was innocuous to animals, but the deposit on the filter remained active. These observations showed, as conclusively as could be expected in the absence of cultivation, that anthrax was caused by a living organism that multiplied in the body, invaded the blood stream, and produced death by septicæmia. To this organism Davaine gave the name of *Bactéridie*—a name by which it is still known among French writers. Subsequently Davaine and Raimbert (1864) found the same organism in a malignant pustule in man, thus demonstrating the ætiological identity of the disease in man and animals.

The final proof of the causative rôle of *B. anthracis* was produced by Koch (1877), who, in a classic masterpiece, which brought him suddenly into fame, gave a full account of the organism, described its formation of resistant spores, its cultivation *in vitro*, the reproduction of the disease by injection of pure cultures, and the recovery of the organism from the animals at necropsy.

The subsequent history of anthrax is largely connected with attempts at

immunization, first made by Toussaint, and later with more success by Pasteur, by Sclavo, and by Sobernheim (see section on Vaccination).

Epidemiology

Anthrax in Animals.—Anthrax is primarily a disease of animals, from which man is secondarily infected. To understand the epidemiology of the disease in man, it is therefore necessary to consider first its incidence amongst animals.

Anthrax is a widespread disease occurring in nearly every country in the world. There are areas, such as the Beauce, Champagne and Auvergne in France, Eastern Prussia, the plains of the Danube, and parts of Siberia and Asia Minor in which it is specially prevalent, and from which it never dies out. As a rule, it is commoner in swampy, low-lying districts with warm, loose, moist soils, and in the great deltas, such as those of the Mississippi and the Brahmaputra. In warm countries its maximum incidence is in the months of June, July, August, and September, but in colder countries, such as Great Britain, the disease is commoner during the winter months. The most susceptible animals are the herbivora. Cattle, sheep, pigs, horses, and goats are the chief sufferers, but the disease may occur in dogs, cats, camels, buffaloes, deer, and even in beasts of prey. Algerian sheep are highly resistant (Chauveau 1880*a*). In Europe the incidence is usually greater among cattle than among sheep, but in Australia and South America sheep are more frequently affected.

Since the Anthrax Order of 1910 came into force, the number of outbreaks in Great Britain has varied between about 400 and 700 a year (Reports 1914–1937). In the year 1937 there were 743 outbreaks affecting 820 cattle, 30 swine, 13 horses, 1 sheep, and 15 other animals. Since the total number of animals affected was only 879, it follows that rarely more than 1 or 2 animals were concerned in any one outbreak. This is very different from the type of outbreak that still occurs in many continental areas, where whole herds may be decimated. A reason for this difference will be suggested under the section dealing with the mode of spread of the disease. Table 165 gives an idea of the frequency of the disease in different parts of the globe (Page 1909).

TABLE 165

SHOWING THE AVERAGE ANNUAL NUMBER OF CASES OF ANTHRAX AMONGST ALL ANIMALS BETWEEN 1902 AND 1906.

United Kingdom	1,263
Italy	4,585
Germany	5,559
British India	29,398
European Russia, Finland and N. Caucasus	46,169

As a rule, anthrax in animals takes the form of a septicæmia, varying from a sudden apoplectic attack with death occurring in a few minutes after the appearance of the first symptoms, to a subacute type, manifested by fever, and frequently by intestinal disturbances, terminating fatally after about a week. In cattle and horses, circumscribed cutaneous swellings or carbuncles may sometimes appear, not unlike the malignant pustule of man; in swine and dogs, localization is common on the mucosæ, particularly those of the pharynx and larynx. These

forms with local manifestations are rarely so fatal as the general septicæmic disease.

Mode of Infection and Spread.—Anthrax is rarely spread directly from animal to animal. Infection generally occurs by the alimentary tract from ingestion of infected food. During the last stages of the disease in animals, the bacilli are excreted in the urine, fæces, and saliva. At the time of death and for some time afterwards, bloody infected fluid exudes from the openings of the body, and soils the neighbouring ground. The bacilli, which in the blood are invariably in the vegetative form, after being voided from the body soon produce spores under the influence of a suitable temperature and free access of oxygen. These spores are extremely resistant to inimical agencies and may remain alive on the surface of the ground for as long as 12 years (Pasteur 1881*a*). Cattle or sheep feeding on this ground are liable to be infected; the spores are taken in by the mouth; they probably pass through the stomach unharmed, multiply in the small intestine, invade the mucosa, and reach the blood stream.

This view, which was largely developed by Koch (1881) differed in some respects from that put forward by Pasteur (1880), who maintained that infection occurred through the mouth and pharynx. As evidence of this, he showed that if sheep were fed on spores, a certain proportion died of anthrax; but that if to the infected food were added prickly substances, such as the pointed extremities of dried thistle leaves or barley spikes, which were able to cause erosions of the pharyngeal mucosa, the mortality amongst the sheep was increased. Pasteur, moreover, maintained that earth-worms played an important part in the actual contamination of the ground. He supposed that, when an animal was buried, the bacilli exuding from the openings of the body developed into spores, which were then brought by earth-worms to the surface of the soil, where they were deposited as casts. It is doubtful what importance is to be attributed to this view. Koch (1881), who examined a number of earth-worms placed in artificially infected soil, found that they rarely became infected; and he brought indirect evidence to suggest that their rôle in the contamination of the ground was negligible in comparison with that caused by the exudation of infected body fluids from animals just before or after death.

The view that the infection of animals occurs chiefly by feeding on contaminated pasture land affords a possible explanation of the greater prevalence of anthrax in low-lying, marshy areas, and by the banks of streams, where grasses and decaying vegetable materials are abundant on which the bacillus may grow; of the rise in incidence as soon as the weather becomes warm; and of the absence of an æstival prevalence of the disease in cold countries. It is, however, by no means clear that the anthrax bacillus can multiply on the surface of the soil under natural conditions, and the view is held by some workers that the organism is an obligatory parasite (see Minett and Dhanda 1941).

In Great Britain, as already stated, the greatest number of cases occurs during the winter months. This fact alone is sufficient to suggest that the mode of infection is different from that in warmer climates. Though occasional infection may occur by contamination of the ground with the effluents from tannery and other industrial works, M'Fadyean (1903*b*) has brought evidence to show that soil infection plays little part in the spread of the disease in this country; and that the majority of cases are traceable to imported food-stuffs. The evidence for this is, briefly, that anthrax occurs sporadically, not epidemically, as might be expected if the soil was the main source of infection; that the disease is commonest during the winter months, when artificial food-stuffs are most used; that it is more frequent amongst cattle, which receive artificial food-stuffs, than amongst sheep, which do not; that 80 per cent. of outbreaks (Stockman 1911)

occur on previously uninfected farms; and that in most outbreaks there is a history of artificial feeding. (See also Jackson 1930.) This evidence, almost entirely circumstantial, has been supplemented by work abroad. In Germany in 1914, there were 7,181 cases of anthrax in animals; in 1919 there were only 743. This tenfold decrease is attributed to the cessation, during the war, of the importation of artificial food-stuffs (Poppe 1922). That such food-stuffs may actually contain anthrax spores has been demonstrated for bone, fish and maize meal, for barley, and for oil-cake.

Another method of infection that may occur, though less commonly, is by flies. *Stomoxys calcitrans*, horse flies, and mosquitoes, for example, have been shown experimentally to convey anthrax (Poppe 1922). The transmission is mechanical (Morris 1918); there is no development of the bacilli in the insect's body.

Summarizing, we may say that in warm countries animals are infected chiefly by contaminated pasturage, less often by biting flies; that in cold countries the chief mode of infection is apparently by artificial food-stuffs; and that the most frequent mode of infection appears to be through the alimentary tract. This last conclusion has been challenged by Besredka (1921) who believes that infection occurs through the skin (see Chapter 53), and by Sanarelli (1925) and by Rovida and Schwarz (1928*a*, *b*), who were unable to demonstrate in laboratory animals the passage of anthrax bacilli through the intact intestinal mucosa.

Excluding very mild and latent cases, the mortality in animals seems to vary between 75 and 100 per cent.

Anthrax in Man.—Anthrax in man may be divided into (1) the non-industrial type, affecting shepherds, farmers, veterinary surgeons, knackers, butchers, pathologists, and others coming into close contact with infected animals; and (2) the industrial type, arising from the manipulation of wool, animal hair and bristles, hides and skins, or occurring in other industries such as those dealing with harness, furniture, cutlery, boots, manure, rag-sorting, horn, or grain porterage. The epidemiology of these two types is so different that they must be treated separately.

The first type, which takes the form of malignant pustule, is due to contamination of the skin with material from infected animals. It is particularly common, therefore, in countries having a high incidence of anthrax amongst animals, and is relatively uncommon in countries, such as Great Britain, where the incidence amongst animals is low. Moreover, there is a close correlation between the seasonal incidence of anthrax in animals and in human beings. This is well seen in southern Italy, where the number of cases in man rises almost simultaneously during the summer months with the increased prevalence amongst animals (Page 1909), and in Bulgaria (Koschucharoff 1938). In the United States agricultural anthrax is on the increase (Report 1934–35).

The second type of anthrax, which may take the form of malignant pustule or pulmonary disease, is dependent on infection acquired during the industrial treatment of animal products, and shows no special seasonal incidence. For convenience of reporting, the cases in this country are divided into 5 groups (Table 166).

Anthrax is a relatively uncommon disease in Great Britain and America. The total number of cases in England and Wales for the twelve years 1913 to 1924 was 743, of which 109, or 14·7 per cent., were fatal. During the twelve years 1927 to 1938 there were only 79 deaths. In the United States during the five years 1934 to 1938 there were 322 cases with 50 deaths, that is, a case mortality of 15·5 per cent. (Reports 1934–38.)

TABLE 166

INCIDENCE OF INDUSTRIAL ANTHRAX IN GREAT BRITAIN.[1]

Year.	Wool.	Horsehair.	Hides and Skins.	Other Industries.	Dock Labourers.	Total.
1913 . . .	43 (5)	5 (1)	19 (2)	3	3	73 (8)
1914 . . .	29 (5)	5	15	6 (1)	7 (2)	62 (8)
1915 . . .	26 (2)	2	18 (3)	3 (1)	0	49 (6)
1916 . . .	80 (12)	6 (3)	18 (3)	2	2 (1)	108 (19)
1917 . . .	65 (11)	1	29 (2)	4 (1)	3 (1)	102 (15)
1918 . . .	49 (4)	4 (3)	14 (1)	1	3	71 (7)
1919 . . .	34 (5)	3 (1)	16 (1)	4 (2)	3 (2)	60 (11)
1920 . . .	25 (7)	5 (1)	17 (3)	1	4 (1)	52 (12)
1921 . . .	11 (3)	4 (1)	8 (1)	2 (1)	0	25 (6)
1922 . . .	19 (3)	9 (1)	16 (1)	1	1	46 (5)
1923 . . .	14 (1)	9 (2)	22 (1)	1 (1)	4 (3)	50 (8)
1924 . . .	19 (1)	4 (1)	16 (2)	4	2	45 (4)
Total . . .	414 (59)	57 (13)	208 (20)	32 (7)	32 (10)	743 (109)

The figures in brackets indicate deaths.

[1] For this Table, we are indebted to the kindness of Dr. S. A. Henry, H.M. Medical Inspector of Factories.

In the woollen industries, anthrax occurs almost entirely round the Bradford district of the West Riding and in Worcestershire; the other centres are exempt. This can be explained by the fact that the dangerous classes of raw wool from Asia Minor and Persia are used in these districts. Amongst those engaged in the horsehair industry, the maximum incidence falls on those using the dangerous classes of hair from China, Russia, and Siberia. Among the dock porters, the incidence falls chiefly on those working in the ports of London and Liverpool (Legge 1905). (For incidence of human anthrax in Germany, Austria, and the United States during recent years, see Report 1934 35.)

In all but the woollen industry, the type of anthrax contracted is the malignant pustule. As infection occurs from contamination of the skin with the infected animal products, it is natural to expect that the uncovered parts of the body will suffer most severely. Legge (1934) gives the following table (Table 167), showing the site of the pustule:

TABLE 167

LOCATION OF MALIGNANT PUSTULE.

Situation.	Number.	Per cent.
Head and face	418	44·6
Neck	292	31·2
Upper extremity . . .	191	20·4
Lower extremity . . .	18	1·9
Trunk	18	1·9
Total	937	100·0

The site of the lesion varies, moreover, with the nature of the industry. Hide porters, for example, are frequently infected on the back of the neck, which is more open than other parts to excoriation. In butchers and others who have to handle

carcasses, the arm is often affected. The face and neck are prone to attack in those using infected shaving brushes (Vincent 1922, Report 1921), and so on. In the woollen industries, infection occurs during the processes of sorting and combing. The worker inhales a large quantity of dust containing anthrax spores, and is hence liable to the pulmonary type of anthrax. Fifty years ago pulmonary infection was commoner than the malignant pustule, but improvements in factory legislation have diminished this type considerably.

The various products that are liable to give rise to anthrax in man come from regions where the disease is common in animals; their danger is in proportion to the chance of their being infected. Hides and wool are frequently contaminated with blood; horse hair and hog bristles are contaminated from various sources.

Owing to their greater exposure, anthrax is more common amongst men than women, but the disease is more fatal in women. The case mortality of cutaneous anthrax depends largely upon the site of infection and the day on which treatment is started. According to Legge (1934) it ranges from about 3 per cent. on the forehead to about 24 per cent. on the neck. Eurich (1930) estimates the usual case mortality at 5 to 15 per cent. Respiratory and alimentary anthrax are almost invariably fatal.

Apart from the methods already mentioned, anthrax may be conveyed to man by the bites of insects that have just fed on the carcass of an infected animal. Contact infection in human beings is uncommon. Occasionally infection may occur by the food, and give rise to the intestinal form. The bacilli are destroyed by the gastric juice, but the spores escape and multiply in the intestine. Experimental evidence, based on findings in laboratory animals, suggests that for this mode of infection to occur in man, large numbers of spores must be present in the food. It is worth while mentioning that the milk of animals dying or just dead of the disease may contain the bacilli (M'Fadyean 1909); chances of infection by this method must, in this country at least, be rare.

It will be seen, therefore, that man is infected with anthrax only as a result of his dealings with animals or with animal products. Methods of prevention must be founded on an understanding of this fact.

Apart from malignant pustule, and the less common respiratory and intestinal forms of the disease in man, meningitis is not infrequently caused by the anthrax bacillus.

Bacteriology of Anthrax in Man and Animals.

Anthrax is a disease that, when fatal, invariably terminates in septicæmia. Whatever form the disease takes—the malignant pustule, respiratory, or intestinal—it is characterized by a primary local proliferation of the bacilli, with the formation of local lesions. The fate of the animal rests on the result of this local attack; if it is resisted by the phagocytes and other defences of the body, recovery rapidly occurs; if the bacilli prove too virulent, they invade the blood stream, and multiply abundantly. This invasion occurs late—generally not more than a few hours before death—and is accompanied by severe toxic manifestations. It is characteristic of anthrax that the bacilli remain confined almost entirely to the blood vessels; they are found in maximum numbers in the capillaries of the liver, lung, kidney, spleen, intestine, and stomach, in smaller numbers in those of the brain, skin and muscle. Their distribution varies, however, with the animal attacked. In the blood of mice or rabbits, for example, there are few bacilli to be

found; in guinea-pigs there may be more bacilli than red blood corpuscles. In the larger animals they are usually plentiful, but pigs and horses may die before the organisms have proliferated sufficiently to be detected microscopically (Stockman 1911).

In man, the malignant pustule starts as a small area of inflammation. It increases in size at a varying rate. Coagulation necrosis of the centre occurs, leading to the formation of a brown, purplish or black eschar surrounded by an intermediate zone of vesicles filled with clear yellow or sanious fluid, and an outer zone of widespread œdema and induration. There is no true pus and relatively little pain (Hodgson 1941). The bacilli are most abundant immediately below the central necrotic area. Invasion of the blood stream may occur, but the number of organisms is seldom as great as in the larger animals (Eurich 1930).

Experimental Production of Anthrax in Animals.—Under natural conditions, the disease is confined chiefly to the herbivora and to man, but occasionally other animals are attacked. Experimentally the disease can be reproduced in the herbivora, rodents, and the omnivora; birds, with the exception of sparrows, and to a less extent hens, ducks, and pigeons, are resistant. Reptiles and fish are insusceptible to infection.

Most of the early experiments on the larger animals were performed with the infected blood of other animals, and it was found that for transmission to be successful certain precautions had to be taken. Putrid blood, for example, often proved non-infective; as did blood that had been rapidly dried after removal from the body. On the other hand, blood or tissues that had been dried slowly were found to retain their infectivity for at least 4 years (Koch 1877). The occurrence of these anomalies was shown by Koch (1877) to be dependent on the presence or absence of spore formation. He found that whenever the bacilli had formed spores, and these spores were capable of being cultivated *in vitro*, the material containing them was infective to animals. The blood of a fœtus removed from a cow that had succumbed to anthrax proved innocuous, showing that the organisms had not passed the placental filter.



FIG. 292.—*Bacillus anthracis.*
Smear from spleen of experimentally infected guinea-pig, showing the bacilli in large numbers (× 1000).

The disease produced in the larger animals is similar to that occurring naturally. Certain points, however, deserve notice. Sheep can be infected equally well by whatever route the organism is introduced. Cattle, on the other hand, are resistant to subcutaneous inoculation, but are easily infected by the alimentary route (Koch *et al.* 1884). Subcutaneous inoculation in cattle may give rise to a severe spreading œdema, but never to a true malignant pustule; moreover, the animal generally recovers. Chauveau (1880*b*) found that the dose was of considerable importance. Large doses injected into sheep caused a 100 per cent. mortality; smaller doses caused a 50 per cent. mortality; whereas tiny doses of about 50–100

organisms were well supported, and were followed by immunity. In feeding experiments, the dose is very important. Oppermann (1906) was unable to infect sheep by this method with less than 100,000 spores. Koch, Gaffky and Loeffler (1884) found that sheep could be infected by feeding as certainly as by subcutaneous injection, provided large numbers of spores were given; with small numbers the results were irregular or completely negative. (For pathogenicity to small animals see p. 844.)

Diagnosis.

In animals suspected of having died of anthrax, it is important that a post-mortem examination should not be made, otherwise blood will be spilt on the neighbouring ground and will provide a source of infection for other beasts. In this country such post-mortems are now illegal. It is sufficient in cattle and sheep to cut off an ear and send it to the laboratory; or alternatively a swab should be soaked in the blood and several blood-films should be prepared for examination. In pigs and horses, however, as death may occur in the early stages of septicæmia when very few organisms are present in the blood, it is advisable to excise a superficial lymphatic gland, and to make smears of any œdematous fluid that may be present. Malignant pustule can often be diagnosed by bacteriological methods early in the disease, but anthrax of the respiratory and alimentary systems can rarely be diagnosed till late.

Microscopical Examination.—Microscopical examination of fresh material is generally sufficient to enable a provisional diagnosis to be made.

The blood or fluid should be spread on a slide and stained without fixation by Gram's method. A rather thicker film should be fixed imperfectly by three passages through the flame, and after being allowed to cool, stained for a few seconds with 1 per cent. aqueous methylene blue, washed thoroughly in water, and dried in air. By Gram's method the bacilli are stained violet; by the second method—described independently by Heim (1901) and M'Fadyean (1903*a*, *c*, 1904)—they are stained sky blue, but are surrounded by a deposit of granular or amorphous material, coloured reddish-purple, probably representing the debris of the imperfectly fixed capsules. The bacilli are arranged singly or in pairs; usually they are about 4–6 μ long, but at times filamentous forms may be seen. They may be closely simulated by *Cl. welchii*, a large Gram-positive capsulated anaerobic bacillus which may invade the blood stream in certain conditions; the exclusion of this organism must rest on cultural examination. Their number



FIG. 293.—*Bacillus anthracis.*

Smear from a malignant pustule in man, showing the bacilli in small numbers (× 1000).

varies greatly. In the fluid taken from a malignant pustule in man, they may be so few as to escape observation; but if sections are made of a portion of excised tissue, embedded in paraffin, and stained by Gram, they can generally be found. Not too much attention should be paid to negative findings.

Cultural Examination.—Where possible, fresh material should be used. The blood or tissue juice may be taken on a swab, but unless it is to be examined at once it is better to collect it on a sterile thread or fragment of earthenware, or a piece of gypsum that has been soaked in broth and subsequently sterilized (Strassburg method). The drying of the material prevents the destruction of the bacilli by the bactericidal power of the serum (Eurich 1933), facilitates their rapid sporing, and prevents the growth of other organisms. Cultures are put up in the usual way on to agar plates and into broth, and the resulting growth carefully studied. It is important not to confuse *B. anthracis* with other members of the aerobic spore-bearing group. To avoid this, it should be remembered that the anthrax bacillus is non-motile, and liquefies gelatin slowly, whereas most of the other members are motile, and liquefy gelatin rapidly. (For the further differentiation of *B. anthracis* from anthrax-like bacilli, see Chapter 35.)

If wool or hair is to be examined, it should be soaked in a weak solution of KOH, incubated for some hours, teased out, heated at 80° C. for 2 minutes, and seeded into 2 per cent. peptone agar, which is then poured into Petri dishes. Particular attention should be paid to the deep colonies, which, in cultures less than 24 hours old, have a characteristic opaque white filamentous appearance with sometimes a nebula at one end (Eurich 1912).

Pathogenicity Tests.—If possible, these should be carried out with the pure cultures that have been isolated. If the original material is used, particularly if this is old, putrid, or contaminated, the animal may die of infection with some organism other than *B. anthracis*. As a routine, the best animals to employ are the mouse and the guinea-pig.

About 0·5 ml. of a 24-hours' broth culture should be injected subcutaneously into the thigh. Death generally occurs within 2 to 3 days; post mortem, there is a hæmorrhagic gelatinous exudate at the site of inoculation, and a large, congested, dark-red spleen (see Chapter 35); microscopically, the bacilli are found in the blood and in smears made from the viscera (Fig. 292, p. 1736). Cultures should be put up for confirmation.

If the only available material is putrid, it is well to inoculate 3 or 4 mice by scratching the skin with a needle dipped in the fluid. As the anthrax bacillus can gain entrance through the abraded skin more easily than other organisms with which it is liable to be contaminated, death of one or more of the mice will probably occur from a pure anthracæmia. This method, first described by Koch, Gaffky and Loeffler (1884), is a good example of the method of purification of cultures by animal inoculation (see Chapter 12).

Wool, hair, or other industrial material should be suspended in water—if necessary after alkaline treatment in the way described under cultural examination—the suspension should be centrifuged, and the deposit inoculated into 2 or 3 guinea-pigs. The animal test is more delicate than the plate method. Using it, Glynn and Lewis (1912), to whom reference should be made for fuller particulars, isolated anthrax spores from 21·3 per cent. of 141 samples of industrial material—mainly hides, wool, hair and bones—supposed to have produced anthrax in Liverpool.

Serological Diagnosis.—Neither the agglutination nor the complement-fixation test is of value for diagnosis. There is one test, however, depending on the presence in infected animal tissues of a substance—a precipitinogen—that reacts with a suitable immune serum to form a precipitate, which has been found of great value; this is Ascoli's thermoprecipitin reaction.

Ascoli (1911) worked with saline extracts of tissues at room temperature, but he found that, for practical purposes, time could be saved by extraction at 100° C. For diagnosis,

the organs or blood of the suspected animal are ground up and suspended in saline, boiled for 5 minutes, filtered through paper and allowed to cool. Of this extract, 0·5 ml. is run very carefully on to the surface of a similar quantity of immune serum in a small tube. Within 15 minutes at room temperature, a definite ring-like precipitate forms at the junction of the two fluids. It is necessary to control the test very carefully with a known anthrax extract and with a normal serum. The method is a delicate one, and may give a positive result with material that has been kept for 2 years or more. A modification of the test was described more recently by Standfusz and Schnauder (1925), and an improved method of preparing high-titre precipitating sera by Rosenberg and Romanow (1929). The precipitable substance in the tissues is derived both from the capsular protein antigen and the somatic polysaccharide antigen. According to Tomcsik and Szongott (1933), most commercial sera contain precipitins only for the polysaccharide. Since there is more protein than polysaccharide antigen in the tissues (Tomcsik and Bodon 1934), it would probably be better to use a serum containing precipitins for both components (see also Iványovics 1939).

Natural Immunity.

The investigation of the natural resistance of certain animals to anthrax played a prominent part in the controversy between the cellular and humoral schools of immunity, in the early days of bacteriology. Without attempting to adduce all the evidence that was brought forward during this controversy, we shall merely draw attention to some of the salient points. Von Behring (for references see Chapter 52) found that the blood serum of the rat—an animal that is fairly resistant to anthrax—possessed a remarkable destructive action on the bacillus and he was led to conclude that the immunity of this animal was dependent on the bactericidal content of its serum (see p. 1173). On the other hand, Metchnikoff and his co-workers stated that, though the blood serum of the rat is bactericidal *in vitro* to the anthrax bacillus, the blood plasma *in vitro* does not possess this action. Furthermore, the spores are able to develop in the body of the rat, and to cause a symptomless infection (Kritschewski and Messik 1930); their further proliferation is restrained by the attack of the phagocytes, which ingest and destroy them. The serum of the rabbit, like that of the rat, has a bactericidal effect on the anthrax bacillus, yet the rabbit is susceptible to experimental inoculation with this organism.

The dog presents another interesting example. When young it is fairly susceptible; when older it is more resistant to experimental injection. Nuttall (1888) found that the defibrinated blood of the dog readily destroyed the bacilli; Metchnikoff was unable to confirm this, but demonstrated a close correlation between the phagocytic activity of the leucocytes and the resistance of the animal. Methods such as removal of the spleen, or intravenous injection of fine wood charcoal, which served to divert the leucocytes, destroyed this resistance.

Pasteur, Joubert and Chamberland (1878) found that the natural resistance of fowls to injection with anthrax could be destroyed by immersing them up to the thighs in cold water; they concluded that the immunity of these birds depended on their high normal temperature (41–42° C.), which interfered with the development of the bacillus. That this explanation was incorrect was shown by Wagner (1890), who found that the bacillus developed readily in the blood and blood serum of fowls outside the body, even at a temperature of 43° C. Further experiments led to the conclusion that the immersion in cold water served to lower the phagocytic response, and thus allow the bacilli to proliferate. We may also quote the experiments of Charrin and Roger (1890), who stated that the natural resistance of rats could be broken down by excessive exercise, and of Preisz (1909), who found that frogs were resistant if kept at 18° C. but not at 30° C.

Weyl (1892) found that anthrax-infected threads, planted in the subcutaneous tissues of hens or pigeons, were rendered avirulent for mice in 4 days; he satisfied himself that the bacilli were killed by the phagocytes as soon as the spores germinated.

Chauveau (1880c) stated that if anthrax bacilli were injected intravenously into vaccinated animals, the bacilli rapidly disappeared from the circulation, being filtered off by the lungs and spleen; these bacilli, however, retained their vitality for some days. Sobernheim (1904) likewise found that the bacilli could live for weeks in the local infiltration produced in immunized sheep without undergoing any loss of virulence. From these experiments, it is clear that a number of bacilli may remain alive and virulent in the tissues without giving rise to disease.

The evidence that natural immunity to anthrax depends on the presence of β-lysins in the blood is unsatisfactory. There is no apparent relationship in different animal species between the presence of these substances and natural resistance to anthrax. It would appear rather that the most important protective mechanism is phagocytosis. Observations are at hand, however, that point to the presence of some other mechanism that prevents the proliferation of the organisms within the body. The nature of this mechanism is at present unknown.

Prevention, Vaccination, and Serum Treatment.

The usual hygienic measures should be adopted on the occurrence of anthrax in a herd. Every precaution should be taken to avoid contamination of the stall or pasture with blood or other fluid exuded from the animal. The carcass should be buried at a depth of 6 feet, or cremated. Other animals in the herd should be watched, and their temperatures should be taken before milking; any animal showing a rise of temperature should be isolated, and its milk withheld from distribution (M'Fadyean 1909).

Where the disease is epidemic, prophylaxis is best assured by vaccination. In countries in which the cases are sporadic, close attention should be paid to the artificial food-stuffs, as these appear to be responsible for a large proportion of the outbreaks.

The prevention of industrial anthrax in man is directed partly to disinfection of the imported animal products, wool, hides, horsehair, and so on, and partly to diminishing the risk of contact with dangerous material in the factories. For the various methods of disinfection, and for factory legislation, the reader is referred to textbooks of Hygiene. Briefly, however, the disinfection of bales and fleeces may be accomplished by preliminary treatment with warm alkali and soap, followed by exposure to a 2 per cent. solution of formaldehyde and drying in hot air (Legge 1934). Robertson (1932) recommends the use of H_2S for the disinfection of hides; anthrax spores are destroyed in 7–16 days.

Vaccination.—In 1879, Chauveau (1880*b*) found that an animal which survived inoculation with anthrax was more resistant to subsequent inoculations. The following year, Toussaint (1880*a*, *b*) successfully vaccinated sheep with defibrinated anthrax blood that had been heated for 10 minutes at 55° C. In 1881, Pasteur (1881*b*, *c*) introduced a method of vaccination, founded on the same principle as that which had been successful in diminishing the virulence of the organism of chicken cholera. He found that if the anthrax bacillus was cultivated in broth at 42–43° C., not only did it lose its power of forming spores, but it gradually decreased in virulence, till after 2 to 3 months it was no longer able to give rise to disease, even in the most susceptible animals. Pasteur prepared two vaccines; the first or *premier vaccin* was a subculture in broth from a strain that had been

kept at 42–43° C. for 15 to 20 days; its virulence was such that it could kill mice and young guinea-pigs, but was unable to kill adult guinea-pigs or rabbits. The second or *deuxième vaccin* was a subculture after 10 to 12 days; it was much more virulent, being able to kill mice, adult guinea-pigs and a certain proportion of injected rabbits.

Arrangements were made with the President of the Agricultural Society of Melun for a trial of this new method under field conditions. Accordingly on May 5, 1881, on a farm at Pouilly-le-Fort, 24 sheep, 1 goat and 6 cows received their first vaccination, and on May 17 they were vaccinated a second time with the more virulent but still attenuated strain. On May 31, Pasteur and his co-workers, Roux and Chamberland, inoculated each of the vaccinated animals with a virulent culture of anthrax; at the same time a series of control animals consisting of 24 sheep, 1 goat and 4 cows were similarly inoculated. Two days later, the vaccinated sheep and the goat were in perfect condition; the control sheep and the goat were all dead. The 4 unvaccinated cows were suffering from severe œdema and fever; the 6 vaccinated cows had neither fever nor œdema. The following day one of the vaccinated sheep died, but at necropsy it was found to be carrying a fœtus that had been dead for about a fortnight.

The success of this experiment led to the vaccination on a large scale of cattle and sheep in Europe, in South America, and in other countries. In 1894 Chamberland collected the statistics that were available for the years 1882 to 1893 inclusive (Table 168).

TABLE 168

RESULTS OF VACCINATION BY PASTEUR'S METHOD.

No. of sheep vaccinated	1,788,677
Mortality after 1st vaccine	5,668, or 0·32 per cent.
Mortality after 2nd vaccine	4,406, or 0·24 „
Mortality during the rest of the year	6,798, or 0·38 „
Total mortality	16,872, or 0·94 „
No. of oxen and cows vaccinated	200,962
Mortality after 1st vaccine	177, or 0·09 „
Mortality after 2nd vaccine	82, or 0·04 „
Mortality during rest of year	432, or 0·21 „
Total mortality	691, or 0·34 „

Before vaccination, the mean mortality of sheep was 10 per cent., of cattle 5 per cent. Chamberland calculated that vaccination had effected a saving of 5 million francs on sheep, and of 2 million francs on cattle.

In Germany, from 1901 to 1908, the total mortality of vaccinated cattle was 0·03 per cent., of horses 0·05 per cent., and of sheep 0·06 per cent. (Klimmer 1911).

At first sight these figures seem impressive, but further consideration suggests suspension of judgment. The method of comparing the mortality in vaccinated animals, during a given period, with the mortality amongst unvaccinated animals in a preceding period, is fallacious; there is no evidence to show that the conditions of the two sets of animals were identical. Large numbers of the vaccinated animals were never subsequently exposed to any risk of anthrax at all; hence no conclusions relative to their immunity can be drawn. Moreover, it would appear that anthrax in France is nearly as prevalent as it was before the vaccination era (Page 1909).

There have been many unfavourable reports on the Pasteurian method of vaccination. One of the chief objections is that it is difficult to standardize the virulence of the two vaccines exactly. Sometimes the virulence is too high, so that large numbers of animals die of the injection alone; sometimes it is too low and confers no protection. Pasteur stated that the decrease in virulence at 42–43° C. occurred gradually, so that at any moment it was possible to fix the virulence at a given point. It is fairly clear now that this statement was incorrect. Preisz (1911), in an illuminating article, showed that in a culture maintained at 42–43° C. bacillary variants appear, some of which possess no capsules and are avirulent, while others possess capsules and are highly virulent; intermediate forms, some spore-bearing, others non-spore-bearing, may appear, and may also differ in virulence. One and the same culture, therefore, may contain both highly virulent and quite avirulent varieties, besides a number of intermediate forms. Moreover, there is no strict relation between the length of incubation and the appearance of the different variants (see Chapter 35). These observations serve to explain the anomalous results of different workers, and show the difficulty of obtaining vaccines of fixed virulence by Pasteur's method.

Nevertheless there are now a few strains, like the Carbozoo and the Boshoff strains, that appear to have become stabilized at a fixed level of attenuation. One of these—the Carbozoo strain—has been used by Mazzucchi (1935) for the preparation of a vaccine in which the spores are suspended in a 2 per cent. solution of saponin; the object of the saponin is to bring about at the site of injection the early formation of gelatinous œdema which acts as a barrier against the rapid dissemination of the bacilli. Field tests with this vaccine have yielded very promising results, and it is now being used extensively in different parts of the world. More recently Sterne (1939) in South Africa has introduced a vaccine prepared by suspending avirulent uncapsulated sporing bacilli in 50 per cent. glycerol saline. Tests on 2½ million cattle and several thousand horses and sheep are reported to have shown that this vaccine gives as good protection as the saponin vaccine, and has the advantage of being safer and producing less reaction.

Reference may be made briefly to Cienkowsky's method, which consists of the use of two vaccines of spore-bearing bacilli attenuated by heat and standardized in virulence by repeated passage through gophers (*Zieselmaus*). It has been used chiefly in Russia, and also in Japan (Poppe 1922).

Sobernheim's Combined Method.—This is a method of combined active and passive immunization. Sobernheim (1902, 1904, 1906) prepared an immune serum by injection of cattle or sheep with cultures of increasing virulence. For his vaccine, he used a culture slightly attenuated by growth at 42·5° C. In practice, simultaneous injections are made, beneath the skin, of 10–16 ml. of immune serum and 0·5–1·0 ml. of vaccine. This method has been extensively adopted in Germany and South America, where it appears to have given good results. It has the merit of requiring only one injection instead of two; of conferring a passive immunity which protects the animal while an active immunity is developing; and of being attended by very little danger. It can be employed after an epidemic has broken out, without fear of rendering the animals more susceptible to infection.

None of the methods in use confers an immunity for more than 9 months or a year; hence vaccination must be repeated annually. It is extremely difficult to gather from the imperfect figures that have been reported what the value of vaccination really is. Instead of vaccinating alternate animals, and comparing

the mortality of the vaccinated with the control animals in the same herd, the experiments have nearly all been made by vaccination of the entire herd, and comparing the mortality of the vaccinated animals with that of the non-vaccinated animals during previous years. The evidence is therefore circumstantial, and cannot be considered satisfactory.

On the whole, however, vaccination does seem to be of value in districts in which the disease is rife. It should never be employed in countries where merely sporadic cases occur, for the living bacilli that are injected may actually help to spread rather than to restrict the disease. Of the various methods referred to, the saponin Carbozoo vaccine seems to enjoy the greatest favour at present for the routine immunization of large animals.

Serum Treatment and Chemotherapy.—Several workers have prepared an immune serum by injection of animals with cultures of increasing virulence, and have found it capable of conferring passive immunity on rabbits. Sobernheim, in particular, has obtained satisfactory results both for protective and for therapeutic purposes with immune sheep and cattle serum, but speaking generally the serum treatment of animals has never been employed on a large scale.

In man, on the other hand, serum treatment is of great importance, and Sclavo's serum appears to have given excellent results. Sclavo (1895) first immunized sheep, but later (1896, 1898, 1901) he found that the ass gave the most satisfactory results. In 1903 Sclavo collected 164 cases of anthrax treated with serum in Italy; there were 10 deaths or a mortality of 6·09 per cent. compared with a mortality of 24·1 per cent. for the whole of Italy (see Page 1909). Legge (1905) states that the mortality of cases treated early varies in different series from 7·1 per cent. to 15·4 per cent., as against 25 per cent. for untreated cases. In this country Eurich (1933) has had a mortality of only 5 per cent. in his last 200 cases of cutaneous anthrax. His treatment consists in never excising the pustule, except when it is in a site, such as the neck, where rapid extension is common, in immobilizing the part, in giving 80 ml. of Sclavo's serum intravenously or subcutaneously on the 1st day and 60 ml. on the 2nd, and in combining serum treatment with a moderately large dose of salvarsan on the 1st and 2nd days. This treatment is similar to that advocated in the United States (Report 1934–35).

Hodgson (1941, 1944) has reported a series of 107 cases of cutaneous anthrax treated without surgical excision of the lesion. Of 52 that were given serum only, 6 died; of 55 that were given serum plus neoarsphenamine (neokharsivan), none died. Serum is given in doses of 100 to 300 ml. each day for five days, with 0·3 gm. of neoarsphenamine on alternate days. Improvement is seldom evident in less than 36–48 hours. (See also Lucchesi and Gildersleeve 1941.)

Cruickshank (1939) found that in the treatment of experimental anthrax in the mouse sulphapyridine was far more effective than arsenical compounds. This conclusion seems to be borne out by Gold (1942), who treated 42 cases of anthrax with sulphonamides. Favourable results were obtained in 39 cases; the other three recovered under subsequent serum treatment. Though sulphapyridine appeared to be most active, Gold regards sulphathiazole as the compound o choice for general purposes. If there is no response within three days, serum should be substituted. It may be added that Murphy, La Boccetta and Lockwood (1944) have reported rapid recovery in three cases of cutaneous anthrax treated with penicillin.

The way in which the serum acts is unknown. It contains neither bactericidins

nor antitoxins; the agglutinin content is said to be no higher than that of normal serum; but Cler (1906) states that it contains opsonins and complement-fixing bodies. Sobernheim (1904) found that immune serum could be heated to 60–70° C., could be kept for 2½ years without any precautions, or could be frozen and thawed, without suffering any diminution of its protective power. Tomesik and Iványovics (1938) and Iványovics (1938*a*, *b*) find that it is possible to prepare two types of sera. One type, which can be obtained by injecting killed encapsulated bacilli into rabbits, contains capsular antibody. The other, which can be obtained by injecting rabbits, horses and certain other animals with living bacilli or with œdema fluid, contains antibody to the somatic polysaccharide substance but little or no capsular antibody. The first type of serum confers passive protection on mice, the second does not. On the other hand, animals producing the first type of serum are not themselves immune, whereas animals producing the second type are highly resistant. All the commercial sera so far examined appear to be of the second type. Which type of serum is of more value in human therapeutics is still doubtful. Until this question is settled, no satisfactory method of standardizing anti-anthrax serum can be proposed.

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CHAPTER 77

TETANUS

History

This disease was described by Hippocrates, Aretæus, and others, but its nature remained obscure till Carle and Rattone in 1884 demonstrated its transmissibility to animals They injected a number of rabbits with a suspension of an acne pustule, which had been the starting-point of a fatal attack of tetanus. The injections, which were made into the sciatic nerve or into the back muscles, were followed in 2 or 3 days by tetanus. The disease could be transmitted to fresh animals by injection of a suspension of nerve tissue.

In the same year Nicolaier (1884, 1886), working at Göttingen, found that the inoculation of earth into mice, guinea-pigs, or rabbits was frequently followed by a disease closely simulating human tetanus. In the pus at the site of inoculation, besides cocci and other organisms, he noticed long, thin bacilli, resembling, but rather larger than, Koch's bacillus of mouse septicæmia (*Ery. muriseptica*). Though he was unable to isolate this organism in pure culture, he grew it in deep coagulated serum for seven generations, and reproduced the disease by injection of the last culture. In animals dying of the experimental disease Nicolaier was unable to find these bacilli microscopically except in the local lesion and, occasionally, in the sciatic nerve sheath and spinal cord. Their limited distribution led him to suggest that the organisms multiplied locally and produced a strychnine-like poison, which, on absorption, reproduced the disease. In 1886 Rosenbach observed a similar bacillus with a round terminal spore in a human case of tetanus, and the pus proved infective to animals. The final demonstration of the ætiological rôle of the tetanus bacillus was furnished in 1889 by Kitasato, who isolated it in pure culture from pus. This he accomplished by heating the pus to 80° C. for 45 to 60 minutes to destroy non-sporing organisms, plating out on gelatin, and incubating in an atmosphere of hydrogen. The inoculation of pure cultures into animals was successful in reproducing the disease.

Epidemiology.

A. Incidence.—Tetanus is an infective disease resulting generally from the contamination of a wound or raw surface, and characterized by a series of tonic muscular spasms usually commencing in the neighbourhood of the site of infection. The frequency with which the masseter muscles are involved has given rise to the popular term lockjaw. In mild cases the spasms may remain localized, but as a rule they become general and involve the whole of the somatic muscular system. In infants the cut surface of the umbilical cord may afford an entrance to the bacilli—tetanus neonatorum; and in mothers tetanus occurs, though very rarely,

as the result of infection in the post-partum genital tract. Infections of the face or head give rise to a peculiar form—cephalic tetanus—characterized by facial paralysis and dysphagia. Visceral tetanus is an uncommon form, in which infection appears to originate from the bacilli in the intestinal tract.

In this country the disease is comparatively uncommon. From 1915 to 1924 the mean annual number of civilian deaths in England and Wales was 157·7, of which 123·2 were in men and 34·5 were in women. But in other countries, particularly the tropics, the disease is more widespread. In 1909 there were 1,373 deaths from tetanus amongst 732,528 deaths from all causes in 18 states of North America; of these 30·7 per cent. were in children under 1 year (Osler 1920). At one time the 4th July celebrations were followed by a large number of cases, usually resulting from blank cartridge wounds (Smith 1908).

Tetanus has followed the administration of gelatin for the control of hæmorrhage, of vaccine lymph, of antitoxic sera, of bacterial vaccines, and the insertion of catgut (Smith 1908).

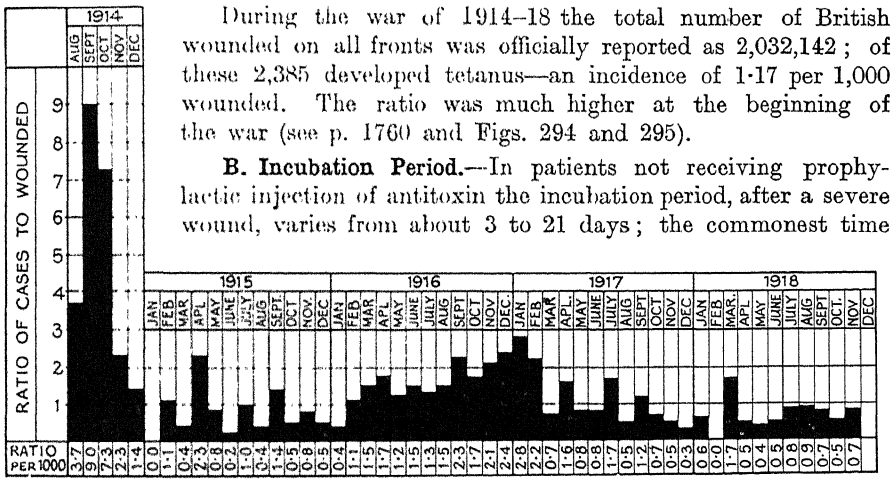


FIG. 294.

The ratio per 1,000 of cases of tetanus to wounded. (After Bruce.)

During the war of 1914–18 the total number of British wounded on all fronts was officially reported as 2,032,142; of these 2,385 developed tetanus—an incidence of 1·17 per 1,000 wounded. The ratio was much higher at the beginning of the war (see p. 1760 and Figs. 294 and 295).

B. Incubation Period.—In patients not receiving prophylactic injection of antitoxin the incubation period, after a severe wound, varies from about 3 to 21 days; the commonest time of onset is about the 7th day. During the war the incubation period increased enormously—partly on account of the use of antitoxin prophylactically; thus in cases treated in home hospitals, the incubation period increased from an average of 11·8 days in 1914 to 50 days in 1918–19 (Bruce 1920).

C. Mortality.—This depends on the nature of the wound, the site of infection, and the treatment accorded. Severe wounds, especially if deep, lacerated, or badly soiled, are characterized by a high mortality. Wounds of the upper extremities are more dangerous than those of the trunk or lower extremities (Bruce 1920). Tetanus neonatorum is very fatal.

The earlier the onset of symptoms, the more likely is death to occur (Table 169) (Bruce 1920).

In pre-serum days the case mortality was about 85 per cent. (Bruce 1920). Bruce (1915, 1916, 1917*b*, *c*, *d*, 1919, 1920) reports on 1,458 cases of tetanus

TABLE 169

SHOWING THE RELATION BETWEEN THE LENGTH OF THE INCUBATION PERIOD AND THE MORTALITY IN TETANUS.

Length of Incubation Period.	No. of Cases.	Case Mortality per cent.
2–10 days	294	58·1
11–22 days	477	35·3
Over 22 days	579	17·3

that were treated in home hospitals during the war of 1914–18. After 1914 almost every patient had received one or more prophylactic inoculations of antitoxin; the majority had also been treated therapeutically with antiserum. The mortality from tetanus of these cases was as follows (Table 170):

TABLE 170

MORTALITY OF CASES OF TETANUS IN THE WAR OF 1914–18.[1]

Year.	No. of Cases.	Died of Tetanus.	Recovered.	Percentage Mortality.
1914 . . .	182	101	81	55·5
1915 . . .	138	78	60	56·5
1916 . . .	451	170	281	37·7
1917 . . .	376	76	294	20·5
1918 . . .	291	73	204	26·4
1919 . . .	20	3	16	15·8

[1] 21 cases dying from causes other than tetanus have been excluded.

Amongst cases of tetanus developing in France or Belgium and treated abroad the mortality was very much higher. In the spring of 1915 amongst 179 cases there were 140 deaths—78·2 per cent. case mortality. Between July and October, 1916, Leishman and Smallman (1917) reported on 160 cases with a mortality of 73·7 per cent. And between November, 1916, and December, 1917, Cummins and Gibson (1919) reported on 376 cases with a mortality of 67·0 per cent. The higher mortality in France was no doubt due to the fact that cases of tetanus with a short incubation period were treated abroad, whereas those in which this period was longer were generally evacuated to England.

D. Distribution of Tetanus Bacilli.

SOIL.—Nicolaier (1884) demonstrated the presence of tetanus bacilli in 12 out of 18 samples of earth. Some of the specimens—from Leipzig and Berlin—had been kept for years. In this country Fildes (1925*a*) examined 70 specimens of soil from waste and cultivated lands, and found *Cl. tetani* 33 times. In Baltimore, Gilles (1937*a*) found toxigenic strains in 9 of 63 samples of street dust.

ANIMAL FÆCES.—Toledo (Toledo and Veillon 1891) demonstrated the presence of *Cl. tetani* in horse and in cow dung. Noble (1915) found it in 18 per cent. of 61 samples of horse fæces examined, but not in any of 21 samples of cow fæces. Sanfelice (1893) demonstrated it in 7 out of 23 samples of normal guinea-pig fæces. Fildes (1925*a*) isolated it from 34 out of 200 samples of normal horse

fæces from London stables. Kerrin (1929) found it in 8 out of 53 samples of horse fæces, 4 out of 21 samples of cow, 6 out of 23 samples of sheep, 17 out of 37 samples of dog, 52 out of 141 samples of rat, and 6 out of 34 samples of hen fæces. Approximately half of the strains proved to be atoxic.

HUMAN FÆCES.—Pizzini (1898) demonstrated the presence of *Cl. tetani* in human fæces; he found it in 3 out of 10 samples from ostlers, and in 2 out of 90 samples from peasants. On the basis of these results, it has been generally assumed that contact with horses strongly predisposes to the carrier condition in man, but we doubt if this assumption is justified. In this country Tulloch (1919–20) found the tetanus bacillus in 5 out of 31 specimens of fæces, and Fildes (1925*a*) only twice in 200 specimens, while Kerrin (1928, 1929) failed to find it once in an examination of 304 samples of adult stools. Similarly Scheunemann (1931) in Germany was unable to isolate it from 50 samples of human fæces. The tetanus bacillus must be ingested frequently with raw food, but the evidence suggests that it is an organism of passage, which does not find a natural habitat in the human intestine.

Reproduction of the Disease in Animals.

Tetanus can be reproduced by the inoculation of pure cultures or of toxin into mice, rats, guinea-pigs, rabbits, goats, sheep, horses, and monkeys. Cats and dogs are more resistant; birds and cold-blooded animals are highly resistant. The most susceptible animal, calculated on the amount of toxin per gram of body weight necessary to prove fatal on injection, is the horse. This is about 12 times as susceptible as the mouse; the guinea-pig is 6 times, and the monkey 4 times, as susceptible as the mouse (von Lingelsheim 1912, Sherrington 1917). On the other hand, the rabbit is twice, the dog 50 times, the cat 600, and the hen 30,000 times as resistant as the mouse (Kitasato 1891, von Lingelsheim 1912). For a description of the lesions produced in laboratory animals reference should be made to Chapter 36.

Mode of Infection in Tetanus.

Vaillard and Rouget in 1892 found that, if tetanus cultures were heated to 65–67° C. for half an hour to destroy the vegetative bacilli and the toxin, the toxin-free spores remaining could be injected in large numbers into a guinea-pig without giving rise to the disease. Microscopical examination showed that the spores did not germinate in the tissues, but were rapidly taken up by the phagocytes, so that in 2 or 3 days they were completely ingested. If, however, the spores were protected from the phagocytes by being wrapped in filter paper, they germinated and gave rise to fatal tetanus. The same results could be obtained by inflicting a trauma at the site of injection, sufficient to cause tissue necrosis or effusion of blood, or by creating a simple fracture. In the damaged tissue the spores were able to germinate, whereas in clean, aseptic wounds they were unable to do so. The presence of other organisms, particularly aerobic, in the wound also permitted the germination of the spores. From these observations it appears that toxin-free spores are innocuous, but that they can be activated by injuring the tissues or by a secondary infection.

These observations were extended by several workers, of whom, for the sake of convenience, we may quote one of the latest. Francis (1924) found that toxin-free spores were rendered pathogenic for guinea-pigs by the simultaneous injection of staphylococci, or of a tissue debilitant such as quinine. He also observed that if either of these agents

was inoculated some time after the injection of the spores, tetanus frequently developed. The result depended on the time elapsing between the two injections. When the spores and the accessory agent were given simultaneously into the same site, all the animals died; when the accessory agent was given into the same site 9 to 30 days after the spores, 11 out of 24 of the animals died; and when the interval was between 30 and 90 days, only 2 out of 20 died. These experiments show clearly that the spores may survive in the tissues for some time. This confirms the work of Vaillard and Rouget (1892), who found that though the spores were rapidly ingested by phagocytes, they were not necessarily destroyed, but might remain latent within the cells, and spontaneously give rise to tetanus some months afterwards (idiopathic tetanus). Francis concluded that in guinea-pigs they nearly all perished within 30 days, but that in mice they remained latent for at least 4 months.

During the war of 1914–18, renewed interest was taken in the mode of infection. Numerous observations showed the presence of the tetanus bacillus in the wounds of men who manifested no symptoms of the disease. Thus in an examination of 100 soldiers not suffering from tetanus, Tulloch (1919–20) found the bacillus 19 times. Observation also showed that tetanus might not develop for weeks or months after the wound had healed, and might then appear suddenly after an operation, perhaps on another part of the body. There is on record a number of cases in which tetanus developed up to 14 years after the presumed introduction of the spores into the tissues; and Bonney, Box and MacLennan (1938) described one case in which tetanus bacilli were isolated from the uterine tissue ten years after an operation which had at the time been followed by a typical attack of tetanus.

Repeating the experiments of previous workers, Tulloch (1919–20) found that toxin-free tetanus spores injected into animals could be stimulated to activity by the simultaneous inoculation of tissue debilitants, such as lactic acid, saponin, and trimethylamine. The results were not always satisfactory; moreover the type of debilitant necessary to prove effective varied with the species of the animal. He then tried the action of *Cl. welchii* and *Cl. septicum* toxins, and found that the addition of a sublethal dose of toxin to tetanus spores enabled the latter to germinate and give rise to a fatal disease.

Bullock (Gye) and Cramer (1919) made an important contribution to the subject when they discovered that the injection of small quantities of ionizable calcium salts together with toxin-free spores invariably led to the development of tetanus. About 2–3 mgm. of $CaCl_2$ was sufficient for a mouse, and about double this quantity for a guinea-pig. Using washed bacilli—but not spores—the same result was obtained whether the bacilli and the salt were injected into different sites at the same time, or into the same site at different times. Further examination led them to conclude that the effect of the calcium ions was not to alter the virulence of the bacteria or the potency of the toxin, but to produce a local change in the tissues at the site of injection. When the calcium salt and the bacilli were injected at different sites, the lesion occurred at the site of injection of the salt; here, it is stated, the organisms were present in large numbers and were not ingested by phagocytes, whereas at the site of injection of the bacilli they underwent lysis and rapid phagocytosis.

It was further observed that soil had much the same action as that of the pure calcium salt. When, however, the calcium salts in the soil were precipitated by sodium carbonate, the watery extract no longer proved effective. They therefore came to the conclusion that one of the factors in allowing the germination of tetanus spores in a wound is the

presence of ionizable calcium salts. Under natural conditions these are frequently introduced with the earth contaminating the wound. The fact that calcium salts are more abundant in cultivated than in waste soil, and that tetanus was far commoner at the beginning of the war, when the fighting was mainly on the highly cultivated soil of Flanders than towards the end, when the soil had largely become waste, led them to suggest that there might be a causal relationship between the diminution of the calcium salts in the soil and the decrease in the incidence of tetanus.

Careful investigations by Fildes (1927) and Russell (1927) suggested very strongly that the germination of tetanus spores in the tissues is dependent chiefly upon the oxidation-reduction potential of the tissues. Histological examination of the tissues of guinea-pigs inoculated with spores revealed the fact that phagocytosis was always incomplete, and could not therefore be held responsible for the failure of the spores to germinate. It was observed that germination never occurred when the heated spores alone were injected; and that when a mixture of spores and earth was injected, germination occurred only in the small areas of necrosis produced by the earth. Calcium chloride was found to produce a widespread necrosis; spores inoculated at the same time germinated over the whole of the necrotic area. Fildes considered that in the normal tissues the O–R potential is sufficiently high to prevent the spores from germinating; but that in areas of necrosis the potential falls to a point sufficiently low to permit of their germination. He would ascribe in fact the same importance to the O–R potential in determining the germination of spores *in vivo* as *in vitro*.

Further observations by Fildes (1929*a*, *b*) and his colleagues (Knight and Fildes 1930, Campbell and Fildes 1931), have greatly strengthened this view. It was found that tetanus spores were unable to germinate in a medium of pH 7·0–7·6 unless the oxidation reduction potential was reduced to Eh + 0·01 volt or below. The Eh of the subcutaneous tissue of the guinea-pig was found to be above this level. If, however, the partial pressure of oxygen in the tissues was lowered by keeping the animals in an atmosphere containing only 7 per cent., instead of the normal 21 per cent., of oxygen, then the Eh was reduced to a level at which germination of the spores in the presence of a mild activating agent such as aleuronat was often rendered possible. (See also p. 1206.)

The observations of Wámoscher and von Vásárhelyi (1933) also support Fildes' explanation. These workers found that one of the most successful activating agents was a small piece of sterile agar. This acts presumably, not by causing an area of focal necrosis in the tissues, but by furnishing a nidus, cut off from the blood supply, in which the Eh is sufficiently low to enable tetanus spores to germinate.

From these observations we may form a conception of the genesis of tetanus in the body. When tetanus spores are introduced into a wound by contamination with earth, horse fæces, or other material, their fate depends largely on the presence or absence of certain accessory factors. Many of these have been described—notably trauma, hæmorrhage, tissue necrosis; chemicals such as lactic acid, saponin, trimethylamine, colloidal silicic acid, and ionizable calcium salts; and biological substances such as the toxins of *Cl. septicum* and *Cl. welchii*. Which of these factors is the more important it is impossible to say; recent work tends to ascribe less importance to mechanical injury, and more to calcium salts and the toxins of other anaerobes. The presence of a suitable accessory factor enables the spores to germinate and multiply in the tissues; it seems probable that this action is dependent on the production by tissue debilitants of an area of necrosis with a sufficiently low oxidation-reduction potential to permit the spores to germinate. In post-operative tetanus the spores may be derived either from infected catgut (see Mackie 1928, Mackie *et al.* 1929, Bulloch 1929), imperfectly sterilized instruments or dressings, or some other source such as the intestinal or the respiratory tract (see Carbone and Perrero 1895, Motzfeldt 1912, Wright 1930).

Absorption and Mode of Action of Tetanus Toxin.

The absorption and mode of action of tetanus toxin has provided a problem of extraordinary interest to experimental workers. The field covered has been so wide that it is impossible to do more than give here an outline of some of the more important experiments. Those who are interested in the subject will do well to consult the papers of Abel and his colleagues (see p. 1753), which give a far more adequate historical survey than we can provide.

If a guinea-pig is injected with a culture of tetanus bacilli, and allowed to develop the disease and die, it is found that scrapings from the site of inoculation are very rich in toxin; in fact, Francis (1924) calculated that the scrapings from a single animal would be sufficient to kill 4,800 mice. On the other hand the blood serum of the dead guinea-pig contains only a minimum amount of toxin, perhaps just sufficient in 1 ml. to kill one mouse; while the organs are completely non-toxic (Rosenbach 1886, Kitasato 1891). Even the central nervous system, in which the toxin might be suspected of accumulating, is harmless on injection into animals (Kitasato 1889, Meyer and Ransom 1903).

The toxin does not appear to be absorbed by the blood to any great extent, because in the first place the toxin content of the blood is very low, and secondly, in order to produce fatal tetanus, more toxin is required when given by the blood stream than when injected subcutaneously; moreover, with a given dose of toxin the incubation period is longer after intravenous than after subcutaneous inoculation.

Bruschettini (1890, 1892) induced tetanus in rabbits by the injection of toxin beneath the skin or into the sciatic nerve; suspensions of the organs were made and injected into fresh animals. The blood and kidneys proved toxic; the lumbar enlargement of the cord was sometimes toxic after subcutaneous, and invariably after intraneural, injection. He concluded that part of the toxin found its way into the blood stream, whence it was excreted by the kidneys; and that part was absorbed by the peripheral nerves and carried to the central nervous system.

In 1897 Marie found that the direct injection into the sciatic nerve of a sub-lethal dose of toxin was sufficient to provoke a fatal attack of tetanus in a rabbit. But if a lethal dose was injected into the forepaw of a rabbit, whose 2nd cervical nerve on that side had been cut, tetanus failed to develop. These observations suggested that the toxin was absorbed by the peripheral nerves and conveyed to the spinal cord; if these nerves were cut, the toxin was unable to reach the cord, and hence tetanus did not occur.

This suggestion was confirmed by Meyer, who found that the sciatic nerve of an animal into whose foot an inoculation had been made was toxic to other animals. Marie and Morax (1902) repeated this with similar results, and added further important information. Thus, they showed that the peripheral nerves can take up toxin only if their nerve endings are intact. The absorption occurs rapidly; for example the sciatic nerve of a guinea-pig which has been injected in the foot is toxic to mice in a little more than an hour after the inoculation. Further experiments suggested that the toxin passed up the axis cylinders in a centripetal current towards the spinal cord. Thus if the sciatic nerve is cut at the popliteal space some time after inoculation of toxin into the calf, the central end rapidly loses its toxicity. If the toxin is injected directly into the lumbar cord, the sciatic nerves remain free from toxin, whereas both the lumbar and the dorsal cords contain considerable amounts.

Meyer and Ransom (1903), using somewhat different methods, came to very much the same conclusions as Marie and Morax. They injected antitoxin into the right sciatic nerve of a rabbit, and then injected toxin subcutaneously into both hind legs. Tetanus developed in 2 or 3 days, but commenced in the left leg; the right leg remained unaffected. In other words, the antitoxin had apparently blocked the passage of the toxin up the sciatic nerve of the right side, and prevented its reaching the cord.

Again, they injected toxin intravenously into a cat, and on the same day injected the

sciatic and femoral nerves with antitoxin; the cat developed general tetanus with the exception of the hind legs which appeared normal. Their experiments seemed to show that the toxin was absorbed by the motor nerves, up which it passed to the spinal cord, where it exerted its effect on the anterior horn cells. If this is so, then the direct injection of toxin into the anterior portion of the cord should be followed rapidly by tetanus. This deduction is correct. Meyer and Ransom, in fact, found that under these conditions the first symptoms of tetanus in a cat appeared in about 3 hours. This seems to explain why, after injection of pure toxin into a limb, there is an incubation period before the disease manifests itself. This period is occupied in the absorption of the toxin by the nerve endings and its passage upwards to the cord. As a rule, the smaller the animal and the less the distance the toxin has to travel along the nerves, the shorter is the incubation period. In the mouse, for example, it is about 12 hours, in the rabbit 18 to 36 hours, in the dog 36 to 48 hours, and in the horse 5 days (Meyer and Ransom 1903). The dose of toxin administered is also a determining factor. The larger this is, the shorter is the incubation period.

It is necessary for the toxin to reach the anterior horn cells of the cord; this it does by passing up the motor nerves. If the toxin is injected into the posterior roots between the ganglion and the cord, tetanus, as it is generally understood, does not occur. Moreover, if the toxin is injected into a part of the body devoid of motor nerves such as the testicles or the peritoneum, a peculiar form of splanchnic tetanus develops, characterized by a longer incubation period, a more rapid illness, and an absence of muscular spasms (von Lingelsheim 1912).

Summarizing, we may say that *the evidence hitherto quoted* suggests that the toxin, when injected into a limb, is absorbed chiefly by the motor nerve endings; thence it passes up the axis cylinders to the anterior horn cells. Not until these have been reached, do symptoms of tetanus appear. At first the contractions are confined to the injected limb; but later, as the toxin diffuses through the cord, the opposite limb and finally the muscles over the whole body become affected. A small amount is probably absorbed by the lymph and carried to the blood stream. From this it is taken up by the motor nerve endings in different parts of the body. Hence the tetanus developing after intravenous injection of toxin is slower in its development, but is generalized from the first.

This conception of the genesis of tetanus has received very severe criticism from Abel (1934) and his colleagues (Abel and Hampil 1935, Abel *et al.* 1935*a*, *b*, Abel, Evans and Hampil 1936, Firor and Jonas 1938, Abel *et al.* 1938, Abel, Firor and Chalian 1938).

Briefly, these workers object to the hypothesis of axis cylinder carriage on the ground that (1) there is no evidence to show that the axis cylinders ever contain tetanus toxin; (2) there is no known mechanism that will explain how the toxin is transported in the axis cylinders, often for long distances, to the central nervous system; (3) the injection of a sub-lethal dose of toxin directly into the sciatic nerve of the dog does not give rise to tetanus, provided measures are taken to prevent the escape of the toxin into the surrounding tissues; and (4) the injection of antitoxin into the sciatic nerve, provided none leaks into the surrounding tissues, does not prevent the development of local tetanus after injection of toxin into the muscles of the leg.

A second hypothesis, which has been held by some workers, that the toxin is carried to the central nervous system by the endoneural and perineural lymphatic vessels, seems to be ruled out on anatomical grounds. There is no evidence to show that the neural lymphatics drain into the cerebrospinal fluid; on the contrary the evidence seems fairly conclusive that they drain directly into lymph glands, which in their turn drain into the thoracic duct.

Discounting these two hypotheses, as well as that of the transference of the toxin

in the tissue spaces of the nerve trunks by molecular forces, Abel adopts the view that the toxin reaches the central nervous system by the arterial circulation. Contrary to the findings of certain previous workers, he has been able to show that, if doses greater than the minimal lethal dose are used, a very high proportion of the toxin injected into the limb of a sheep may be demonstrated in the lymph-glandular and vascular systems. Minimal lethal doses of toxin are fixed by neural and other tissues, so that the toxicity of any tissue for experimental animals into which it is injected results entirely from the presence of blood or lymph containing free toxin. Secondly, Abel believes that tetanus toxin has a dual action on the central and on the peripheral nervous system. By means of multiple injections at various points of a limb, he has produced local tetanus in dogs with amounts of toxin so small that generalized tetanus never develops. Intravenous toxin, on the other hand, produced both the local muscular rigidity and the reflex excitability of the muscles that characterizes generalized tetanus; while the application of toxin directly to the cord in the region of the anterior horn after a latent period of one to two hours produced the reflex excitability alone. The abolition of local tetanus by section of the appropriate nerve depends, not on the destruction of the path by which the toxin reaches the anterior horn cells, but on the abnormally low tonicity of the motor end-organs.

The hypothesis of a dual action is supported by the work of Harvey (1939) and Schaefer (1944). Harvey found that the intramuscular injection of sub-lethal amounts of toxin in divided doses produced local tetanus not only when the nerve was intact but also when the nerve was cut, *provided that* degeneration had not occurred. Section of the nerve diminished the rigidity, but did not abolish it. From a consideration of the electrical activity in tetanic muscle and the response to curarine, Harvey concluded that the integrity of the nerve ending, not its connection with the C.N.S., was essential for the genesis of local tetanus. He suggested that the toxin might act by increasing the normal leak, and in consequence the synthesis, of acetyl-choline in the end-organs, and by interfering with choline-esterase production. From similar considerations, Schaefer supports the notion of local action by the toxin, and claims to have demonstrated a raised acetyl-choline content in tetanic muscle. He found no evidence of any interference with choline-esterase by the toxin.

The establishment of a dual action of the toxin, on end-organs and on the anterior horn cells or their synapses, would not necessarily support Abel's general hypothesis of an exclusively blood-borne intoxication in tetanus, though it would clearly remove one of the outstanding objections to it. Another objection, made by Friedemann, Zuger and Hollander (1939*a*), is based on experiments indicating a selective impermeability of the capillaries and the central nervous system to tetanus toxin, which they attribute to a peculiarity of the toxin molecule. The toxin must, of course, pass through capillary walls in other tissues, otherwise on any hypothesis intravenous injection of toxin would be without ill effect. There is evidence that the capillaries of the brain may differ from those of the rest of the body in their permeability to various circulating dyes (see Friedemann 1942), but its applicability to the problem of tetanus toxin is contested (see, for example, Doerr 1940). In this connection we may note that Barnes and Trueta (1941), who immobilized the limbs of rabbits in plaster, found that several lethal doses of toxin could be injected into such limbs without ill effect. When after some days the plaster was removed tetanus followed. If we assume an intact blood circulation, it appears that toxin was not absorbed into the blood capillaries; the restoration of movement in the leg might have promoted absorption by the lymphatics, or the diffusion of the toxin to large numbers of end-organs throughout the muscle.

The observations of Friedemann and his colleagues (Friedemann, Zuger and Hollander 1939*a*, Friedemann, Hollander and Tarlov 1941) on the genesis of tetanus in the guinea-pig go far to restoring the position attacked by Abel.

They found that the amount of circulating antitoxin which protected an animal from death was determined by the route of injection of the toxin. With intramuscular toxin, this amount was up to 80 times as great as that needed when the same dose of toxin was given intravenously; but if the motor nerves supplying the injected part were cut, the protective amount of antitoxin was the same for both routes of injection, suggesting that in the intact animal, a considerable amount of toxin reached the central nervous system by some route other than the circulation. They noted that fatal tetanus followed the local injection of toxin in animals whose circulation contained, up to the time of death, a large excess of antitoxin; and, by transection of the cord, were able to limit the tetanus, induced by injection of toxin into one hind limb, to structures innervated from the cord below the level of section. Moreover, in transected animals, tetanus occurred in both hind limbs, due apparently to a migration of toxin across the cord below the level of transection.

Summarizing, we may say that we are still ignorant of the exact mechanism by which tetanus toxin is absorbed, and of the way in which it exerts its action on the body cells. The two main hypotheses are (1) that the toxin is absorbed by the motor nerve endings, and carried up the axis cylinders to the anterior horn cells of the central nervous system, which are then stimulated to produce muscular contractions; (2) that the toxin acts locally on the motor nerve endings, and centrally on the anterior horn cells, which it reaches via the lymphatic vessels and the blood stream.

One difficulty in deciding between these hypotheses lies in the variety of experimental animals—cats, dogs, guinea-pigs—investigated. What is true for the rabbit and guinea-pig may not hold for the cat or the dog. Some of the data upon which the first hypothesis was based are capable of other interpretations, and the hypothesis of dual action merits serious consideration. There are nevertheless certain facts which, if confirmed, cannot be reconciled with the second hypothesis.

With regard to the main assumption in the first hypothesis, the transmission of toxin along the axis cylinder, little can profitably be said. There is no evidence of a protoplasmic flow in the axis cylinder by which toxin could be carried, and it seems likely that movement by diffusion, especially of a substance known to be readily fixed to neural tissue (see p. 1756), would be exceedingly slow. In support of a ready movement of toxin along the axis cylinders it is customary to cite the transmission of neurotropic viruses like poliomyelitis. The analogy must be used with caution, for toxin lacks at least one important property of viruses—namely the power to multiply within the nerve cell itself. Nevertheless, if the available evidence points to the occurrence of axis cylinder carriage, we must not allow our ignorance of the way in which such transmission is effected to prevent us from drawing the logical conclusions.

Immunity to Tetanus.

Natural.—Mammals vary in their susceptibility to tetanus; some, such as the mouse, the monkey, and the horse are highly susceptible; others, such as the dog and the cat, are much less susceptible. Most birds and cold-blooded animals are extremely resistant. Examination of the blood of susceptible and partially susceptible animals shows that, while the majority of cattle contain more than 1/500 unit of antitoxin per ml., no antitoxin is present in the blood of man, horses, dogs, pigs, monkeys, or rodents. Sheep and goats may contain small quantities (Coleman 1931, Coleman and Meyer 1926, Ramon and Lemétayer 1934, 1935). The frequent

presence of antitoxin in the blood of ruminants may be causally associated with the comparative resistance of these animals to tetanus. It is suggested that in ruminants tetanus bacilli multiply and form toxin in the digestive reservoirs which precede the true stomach, and that the toxin so formed, partly modified perhaps by the products of bacterial fermentation, is absorbed and gives rise to the production of antitoxin. Examination of the blood of naturally resistant animals has proved it to be devoid of antitoxin (Vaillard 1892); hence their immunity cannot be attributed to the presence of this antibody.

Marie (1897) injected a rabbit intravenously with 1,000,000 mouse M.L.D. of toxin. Blood was removed at frequent intervals, defibrinated, and injected in 1 ml. quantities into mice. For the first 17 hours the blood proved toxic, but after this time it became innocuous; the toxin had disappeared therefore from the circulation in less than a day. Vaillard (1892), on the other hand, who injected a hen with 20 ml. of toxin, found that it persisted in the blood stream for several days. In the susceptible rabbit the toxin is supposed to be absorbed from the blood by the motor nerve endings, and carried to the cord, where it gives rise to disease. In the non-susceptible hen the toxin does not appear to be absorbed at all, or only slowly; hence tetanus fails to develop.

An interesting contribution to the study of natural immunity was made by Wassermann and Takaki (Wassermann 1898, Wassermann and Takaki 1898), who found that the brain, and to a less extent the spinal cord, of normal animals was able to neutralize tetanus toxin. A mixture of toxin and brain suspension proved harmless on injection into animals. The amount that could be neutralized depended partly on the animal; a guinea-pig's brain, for example, could neutralize completely 10 M.L.D. of toxin, and incompletely 60 M.L.D. They found moreover that the neutralization could occur in the body; a mouse injected with a brain suspension, and 24 hours later given 3 to 5 M.L.D. of toxin, did not develop tetanus. From these experiments they concluded that the brain contained natural antitoxin.

As was to be expected, a number of workers immediately directed their attention to this interesting phenomenon (Marie 1898, Metchnikoff 1898*a*, *b*, Roux and Borrel 1898, Danysz 1899, Marie and Morax 1902). They all confirmed the original observation of the *in vitro* neutralization of toxin by a suspension of brain tissue, but differed from Wassermann and Takaki in their explanation of it. It was found that the cells of the cerebral cortex were most active; that the brain of mammals was more powerful than that of birds and cold-blooded animals; that the neutralizing agent was insoluble in saline; that the union between the toxin and nerve cells was not permanent; that there was no actual destruction of toxin; and that for neutralization to occur there must be an intimate contact between the toxin and the cells. Protection experiments failed completely unless the two substances were inoculated at the same site. It is clear, therefore, that the brain does not act by virtue of its antitoxin content. The nervous tissue of the susceptible mammal has a strong affinity for tetanus toxin and, by combining with it, prevents its transference to the motor cells of the inoculated animal. On the other hand, the nervous tissue of the resistant hen, frog, and turtle (Metchnikoff 1898*a*) has little or no affinity for the toxin, and hence fails to anchor it. That this is the true explanation seems apparent from Roux and Borrel's work (1898), which showed that the lethal dose of toxin given directly into the brain of a rabbit was smaller than that necessary when given subcutaneously. The brain tissue of a susceptible animal resembles antitoxin in combining specifically with toxin, but with fatal effects to the animal concerned.

These observations also explain the fact that the central nervous system of animals dying of tetanus may appear to be devoid of toxin, when in reality it contains a considerable amount. To demonstrate the presence of the toxin it is only necessary to grind the brain up in 10 per cent. saline and to leave the suspension for some time, when the toxin will break away from the disintegrated cells and appear free in the fluid (Tizzoni and Cattani 1891*a*).

Acquired Immunity.—One of the most important advances in the progress of bacteriology was made by Behring and Kitasato (Behring and Kitasato 1890, Kitasato 1891, 1892, Behring 1892), when they discovered that the injection of a filtered culture of tetanus bacilli into an animal stimulated the production of an antibody which was able to neutralize the toxin and to preserve the life of animals injected with it. It protected not only against injections of toxin, but also against inoculation of the tetanus bacillus itself. Thus, if 0·2 ml. of an antitoxic rabbit's serum was injected intraperitoneally into a mouse, and virulent tetanus bacilli were injected subcutaneously 24 hours later, the mouse remained perfectly well. The nature of the union between toxin and antitoxin has been discussed elsewhere (see Chapter 46). We may here confine ourselves to a consideration of its protective action in practical prophylaxis.

The antiserum acts, not by interfering with the germination of spores, nor with the multiplication of the bacilli in the tissues, but by neutralizing the toxin as fast as it is formed. If antitoxin is present in the circulating blood and lymph, it will neutralize the toxin before the latter has time to gain access to the nerves. If the antitoxin is not given till after the formation of toxin has begun, it will be too late to prevent the absorption by the nerves.

It follows that there are rigid conditions governing the usefulness of antitoxin. The most important of these is that it must be allowed to come into intimate contact with the toxin. Unless it does so it is valueless. It is known that the nervous tissue of mammals has a strong affinity for tetanus toxin ; it has no such affinity for tetanus antitoxin. Once the toxin has gained entrance to the nerves it can no longer be neutralized, unless the antitoxin is itself injected into the nerves. Meyer and Ransom (1903) injected antitoxin in large quantities intravenously into a cat, and at the same time injected a dose of toxin into the sciatic nerve ; the cat developed tetanus. The nerve tissue acts as a barrier separating the toxin from the antitoxin ; and nothing short of enormous doses of antitoxin in the blood stream can save the life of an animal if once the toxin has gained access to the nerves. We have, in fact, the anomaly of an animal, whose blood contains sufficient antitoxin to save the life of hundreds of mice, itself dying of tetanus. If the antitoxin is injected directly into the central nervous system, there is some evidence that it is able to neutralize the toxin and prevent further spread of the disease (Roux and Borrel 1898). Roux and Borrel's interpretation of their findings is open to question (see Abel and Hampil 1935), though in more carefully controlled experiments Friedemann, Hollander and Tarlov (1941) demonstrated the superiority of intraventricular over intravenous antitoxin in preventing local tetanus. Roux and Borrel also showed that an actively immunized rabbit, capable of withstanding large doses of toxin subcutaneously or intravenously, yet succumbs as readily to the intracerebral injection of toxin as a normal rabbit.

It should be noted that Abel (Abel *et al.* 1938, Abel and Chalian 1938) distinguished two stages in the action of toxin—adsorption to the nerve cells, and fixation. Fixation coincided with the appearance of tetanic symptoms. In the

sheep, antitoxin given intravenously after demonstrable absorption of lethal amounts of toxin by the nervous tissue, but before symptoms of descending tetanus had appeared, had a curative action.

Diagnosis.

The bacteriological diagnosis of tetanus is often difficult and sometimes impossible.

Microscopical Examination should be made where possible of the pus in the wound, and special note taken of bacilli with round, terminal spores. The presence of " drumstick " bacilli is *not*, however, pathognomonic of infection with *Cl. tetani*. The organisms may be present in such small numbers that they may be altogether overlooked.

Cultural Methods are of more importance. The pus or wound scrapings are seeded into cooked meat medium, or into heart or liver digest broth containing a small portion of sterile rabbit kidney. After 5 days' anaerobic incubation at 37° C., if the typical bacilli are visible microscopically, the culture is heated to 70–75° C. for 20 minutes to destroy non-sporing bacilli, and plated out on liver or serum agar. To obtain a pure culture repeated platings may be necessary. Fildes (1925*a*) has drawn attention to the rapidly-spreading, effuse, proteus-like growth that occurs on an agar slope if the water of condensation is inoculated; the bacilli grow over the whole slope and present a tenuous, jagged, fern-like edge at the top. From this a subculture may be made into the condensation water of a fresh tube, and a pure culture obtained after two or three transfers. Besides studying the cultural and biochemical reactions of the strain it is well to test it with a polyvalent agglutinating serum prepared against the common serological types of bacilli in the neighbourhood (Fildes 1925*b*). Enrichment of the material for culture in broth containing 1/100,000 crystal violet is said to be effective (Gilles 1937*b*).

Animal Inoculation.—Material from the wound should be suspended in saline and injected subcutaneously into mice or guinea-pigs. Though this procedure may give a positive result, it is not advisable to rely on it exclusively. Every effort should be made to obtain the organisms in pure culture before injection; it is generally agreed that animal inoculation from a pure culture is more likely to be successful than from a mixed culture (McCoy and Bengtson 1918, Tenbroeck and Bauer 1922). The signs of tetanus in an animal are almost unmistakable, but a careful microscopical examination of the wound and the blood should be made to exclude the presence of other organisms. If a pure culture of the bacillus gives rise to the disease, then a broth filtrate should be injected into two mice, one of which has been given a prophylactic dose of antitoxin. In this way the toxicity of the strain can be established.

Prophylaxis and Treatment.

The surgical treatment of wounds that are likely to be followed by tetanus lies outside our province, but clearly everything must be done to avoid the persistence of necrotic tissue, and to control bacterial proliferation within the wound. Attention must be drawn to the desirability of using properly prepared and disinfected catgut in the suturing of tissues at operation. The way to ensure this is described in the admirable monograph by Bulloch (1929) and his collaborators Lampitt and Bushill (1929).

Preparation, Standardization and Properties of Antitoxin.—The **preparation** of tetanus antitoxin, which plays so important a part in the prophylactic treatment of tetanus, has been the subject of research ever since its discovery. The pure toxin is too active to be injected directly into susceptible animals, and some method of decreasing its toxicity has to be employed.

Behring and Kitasato (1890) and Kitasato (1891) found that the injection into rabbits and mice of tetanus toxin, modified by treatment with a solution of iodine tri-chloride, rendered them immune; their serum was found to contain an antitoxin capable of neutralizing the toxin. Later Behring (1892) immunized sheep and horses, and was thus able to obtain antitoxin in considerable quantities. Experiments on mice infected with splinters of wood dipped in a heated broth culture of *Cl. tetani* showed that the antitoxin was capable of protecting them if given before, simultaneously with, or 24 hours after the infection. The therapeutic dose required was very much greater than the prophylactic dose. Thus in one experiment the effective dose of serum was 0·001 ml. when given 15 hours before, 0·1 ml. when given at the same time as, and 0·4 ml. when given 24 hours after, the infection (Kitasato 1892).

Vaillard (1892) confirmed the value of iodized toxin, and succeeded in immunizing rabbits by the injection of successive doses of filtered cultures heated at progressively lower temperatures; finally the animals were able to withstand the intravenous injection of pure toxin. He likewise confirmed Behring's and Kitasato's work on the neutralizing and protective powers of the antitoxin, but was unable to satisfy himself of its curative power (Vaillard 1891). After a dose of 0·5 ml. of antitoxic rabbit serum into a mouse, the animal resisted the injection of toxin during the next few days; but after about a fortnight the passive immunity wore off and the animal regained its susceptibility to toxin. Serum injected some hours after the commencement of symptoms was without effect on the progress of the disease. Tizzoni and Cattani (1891*a*) were also unable to convince themselves of the curative power of antitoxin.

Antitoxin was later produced on a large scale by the injection of horses with toxin-antitoxin mixtures (Buxton and Glenny 1921). The toxin was over-neutralized, and a series of injections was given at intervals of a few days over a period of some months. Of recent years the preliminary treatment of toxin with formol has been widely adopted. The action of the formol is to deprive the toxin of its toxicity, while having little effect on its antigenic value. After a series of initial injections with this formolized product—sometimes referred to as anatoxin or formol toxoid—crude toxin itself can be injected. The actual technique of preparing the toxoid need not detain us here. Usually about 0·4 per cent. of formol is added to the toxin, and the mixture is incubated at 37°–39° C. till it is sufficiently detoxified. About 20 ml. of formolized toxin should prove non-toxic to the guinea-pig on subcutaneous inoculation (Hosoya *et al.* 1931, Wilcox 1934). Some workers precipitate the toxoid with alum or zinc chloride, while some add tapioca or 0·5 per cent. $CaCl_2$ in an endeavour to increase its antigenic efficiency (see Glenny *et al.* 1926, Ramon *et al.* 1931, Ramon and Lemétayer 1932, Bergey 1934).

The **standardization** of tetanus antitoxin has presented considerable difficulties, due in part to the liability of tetanus toxin to deterioration. In the past three different units have been employed—a German, a French, and an American. Comparative observations made under the auspices of the Permanent Committee on Standardization of the Health Organization of the League of Nations showed that the relation of these units was as follows: 1 German unit = 66 American = 3,750 French units (see Prausnitz 1929). It was agreed that an international unit should be established equivalent to one-half the existing American unit, and that the standard antitoxin should be kept at the Serum Institute, Copenhagen. So defined, the unit of tetanus antitoxin represents the specific neutralizing activity for tetanus toxin contained in 0·1547 mgm. of the standard preparation. Antitoxin titrations

are best carried out by the subcutaneous injection of toxin-antitoxin mixtures into mice, and the determination of that amount of toxin which just fails to produce tetanus by the 4th day (L0 dose). The L+ dose, *i.e.*, that dose which kills the animal on the 4th day, is much less satisfactory as a basis for standardization.

Comparative tests in a number of institutions have shown that antitoxins can be assayed with some success provided that one specimen of toxin is employed. The toxic filtrates commonly used for assay, however, vary greatly with the strain of *Cl. tetani* and the medium selected for its growth. Even when the standardization of the toxin, and the methods of administering toxin and antitoxin to a given breed of test animals, have been carried to practicable limits, there are qualitative as well as quantitative differences among antitoxins that may vitiate the assay. (Report 1938, Smith 1938, Ipsen 1940–41.)

The potency of proteolytically refined antitoxins may be measured by flocculation methods analogous to the Ramon titration of diphtheria antitoxin (see, for example, Goldie, Parsons and Bowers 1942).

Properties.—Tetanus antitoxin remains fairly stable in a cold, dark place. If dried, powdered, and preserved in vacuum tubes in the presence of phosphorus pentoxide, and kept in the dark at 5° C., it remains unaltered for years (Rosenau and Anderson 1908). Exposure to sunlight or diffuse daylight gradually destroys it. It resists heat at 60° C. for half an hour, but is partly destroyed at 65° C., and completely at 68° C. (Tizzoni and Cattani 1891*b*). It is non-dialysable, and is precipitated by ammonium sulphate with the globulin fraction. Hydrochloric and lactic acids, and caustic potash, destroy it.

Injected intravenously into animals, antitoxin circulates in the blood for some days. A very small amount may pass into the cerebrospinal fluid and into the milk of lactating animals (von Lingelsheim 1912). After subcutaneous injection the highest titre of antitoxin in the blood is reached in 1 to 3 days; from then on it diminishes till after a fortnight only a trace is left. Injected into the sub-arachnoid space it passes almost completely into the blood stream.

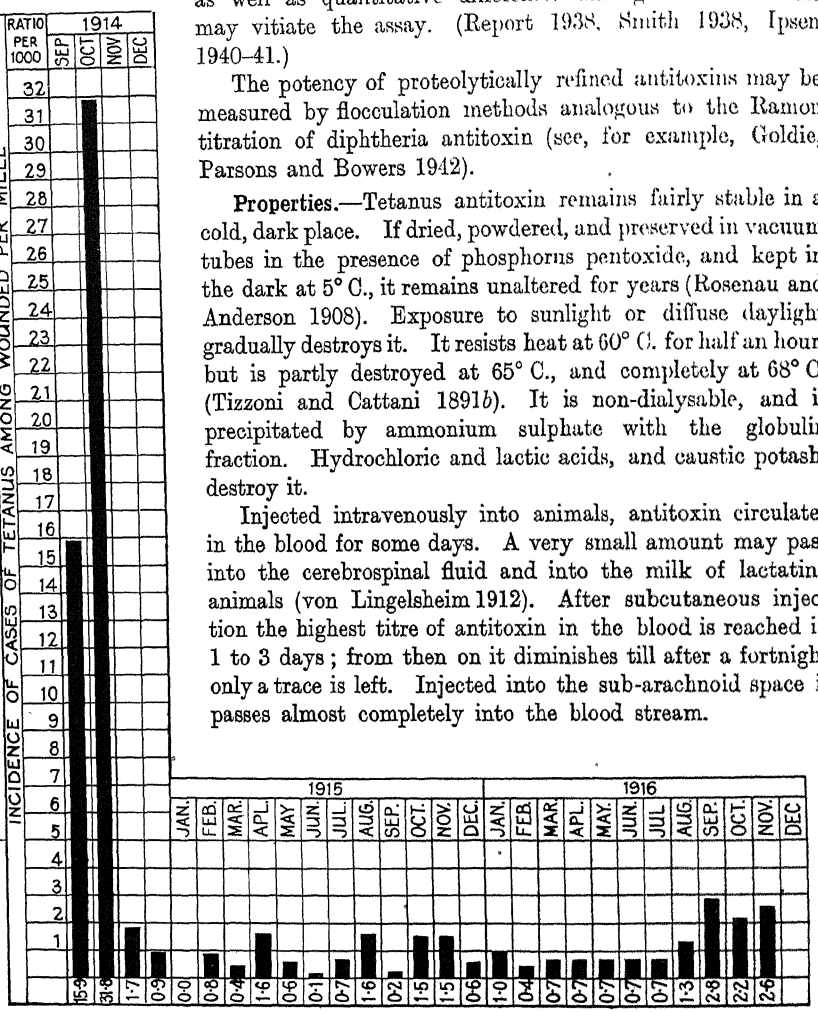


FIG. 295.

The incidence of tetanus per 1,000 wounded in Home Hospitals during 1914–16, showing the fall in incidence after Oct., 1914, when the use of antitetanic serum became generalized. (After Bruce.)

MacConkey and Homer (1917) found that the passive immunity conferred on guinea-pigs by the subcutaneous injection of 3½ units of antitoxin disappeared in about a fortnight. Increased dosage up to 40 units did not prolong this period; but when enormous doses were given—250 to 3,500 units—the animal remained immune for about a month. That is to say, passive immunity is at the best transient, and can be relied upon only for a short time.

Prophylactic Injection of Antitoxin.—In the prophylaxis of tetanus in man it is advisable to inject 500-1,500 units of antitoxin into the subcutaneous tissues as soon as possible after the receipt of the wound. The type that calls for this treatment is the dirty lacerated wound, contaminated with street mud, earth, or animal fæces, especially if accompanied by severe bruising of the tissues or fracture of the bones. It is advisable to inject a portion of the serum in two or three places around the actual lesion. The wound itself should preferably be excised: if this is impossible it should be cleansed and thoroughly drained. If the wound is severe and is likely to take some time to heal, it is well to repeat the injection every week. Should symptoms of tetanus develop, large doses—30,000 units or more must be given every day, partly around the wound and partly intrathecally.

From animal experiments there is not the least doubt of the protective value of antitoxin, but in man the evidence is necessarily indirect. This may be summarized as follows:

(1). The prophylactic injection of antitoxin within a few hours of the receipt of a wound appears to diminish greatly the chances of tetanus. The incidence during the war of 1914-18 is recorded in Figs. 294 and 295. The sudden fall in November, 1914, can in all probability be ascribed to the prophylactic use of antitetanic serum that was introduced about the middle of the preceding month. The rise towards the end of 1916 may have been fictitious; Bruce ascribes it to improved diagnosis, particularly of local tetanus, which was largely overlooked at the beginning of the war. The subsequent fall in 1917 and 1918 may perhaps be related to the more efficient prophylactic serum treatment which was commenced in June, 1917, but more probably to the practice of early excision of wounds which was introduced about the same time.

(2) When it does not prevent the development of tetanus it appears to lengthen its incubation period. This will be seen from Table 171, showing the average incubation period amongst wounded treated in home hospitals during the war.

TABLE 171

SHOWING THE AVERAGE INCUBATION PERIOD OF TETANUS IN DIFFERENT YEARS OF THE WAR.

Year.	Length of Incubation Period in Days.
1914	11·8
1915	27·3
1916	34·0
1917	48·0
1918–19	59·0

A direct comparison of the average incubation period in patients who have received, and those who have not received, a prophylactic injection of antitoxin after having been wounded leads to the same conclusion; in the inoculated this period is 45·5 days, in the uninoculated 10·9 days. In this connection it must be remembered that the passive

immunity conferred by a single injection of antitoxin wears off in about a fortnight or three weeks. During this period the patient is unlikely to develop tetanus. But once this is past, he is probably just as susceptible as a person who has never been inoculated at all. Hence if tetanus bacilli are still present in the wound, they are liable to give rise to disease. If all patients had been given repeated doses of antitoxin at intervals of a week till their wounds healed, it is possible that these late cases of tetanus would have been greatly reduced in number.

(3) Tetanus developing in persons who have received a prophylactic injection appears to be less fatal than in uninoculated persons (Bruce 1920). This is seen from Table 172.

TABLE 172

SHOWING THE MORTALITY OF TETANUS IN INOCULATED AND UNINOCULATED PATIENTS.

	No. of Cases.	Died.	Case Mortality.
			Per cent.
Inoculated.	899	203	22·6
Non-inoculated or unrecorded	559	298	53·3

It is interesting to note that the average mortality amongst the inoculated during the war fell far more rapidly than amongst the uninoculated. This can probably be accounted for by the more regular administration of serum, and by its repeated administration every 7 days for 4 weeks which was undertaken at the instigation of the Tetanus Committee in June, 1917.

(4) During the war the proportion of cases of local to general tetanus rose considerably (Bruce 1920) (Table 173).

TABLE 173

SHOWING THE PROPORTION OF CASES OF LOCAL AND GENERAL TETANUS.

	Percentages.				
	1914.	1915.	1916.	1917.	1918.
General	98·9	98·6	87·0	76·6	83·5
Local	1·1	1·4	13·0	23·4	16·5

This may be in part attributed to the more general and efficient use of antitoxin prophylactically, and partly to improved diagnosis of local tetanus.

Later records of effective antitoxin prophylaxis are scanty and can be considered as no more than suggestive. Thus Wildegans (1940) observed in the Polish campaign of 1939 that the incidence of tetanus among Polish soldiers, over half of whom had received no prophylactic antitoxin, was 66/10,000, as compared with one of 3·6/10,000 in the German soldiers, the majority of whom had been immunized.

Therapeutic Injection of Antitoxin.—Whether or not antitoxin is of any value in man when given subsequently to the development of tetanus is a question that has received no definite answer. The figures collected during the war of 1914–18 were ambiguous, and we see no purpose in retailing them; for their examination the reader is referred to Bruce (1915, 1916, 1917*a*, *b*, *c*, *d*, 1919), Dean (1917), and Leishman and Smallman (1917). Experiments with the smaller animals have shown that it is difficult or impossible to interfere with the progress of the disease once

the first symptoms have manifested themselves, unless enormous doses are given; even then the issue is uncertain.

On monkeys, however—chiefly *Macacus rhesus* and *Callithrix*—Sherrington (1917) obtained perfectly unequivocal results. He injected batches of 25 with 8 M.L.D. of toxin into the outer head of the gastrocnemius muscle. From 47 to 78 hours later, when the early symptoms of tetanus had appeared, he injected 2,000 U.S.A. units of antitoxin per kilo of monkey, giving the serum by different routes to the separate batches. All the control animals receiving no antitoxin died; the mortality amongst the inoculated was as follows (Table 174).

This experiment answered two questions. It showed first of all that antitoxin might be of considerable therapeutic value; and secondly that the route by which it was given was of supreme importance. Whereas antitoxin administered subcutaneously or intramuscularly had but little effect, given intrathecally it saved the lives of over half the animals.

TABLE 174

Serum given by:	Deaths.	Recoveries.	Percentage Mortality.
			Per cent.
Subcutaneous route	23	2	92
Intramuscular route	22	3	88
Intravenous route	18	7	72
Lumbar intrathecal route . .	11	14	44
Bulbar intrathecal route. . .	7	13	35
Cerebral subdural route . . .	10	0	100

It is difficult to explain the discrepancy between Sherrington's experimental results and the results in clinical practice; but two points may be noticed. Firstly the monkeys were injected with pure toxin, not with bacilli; it is doubtful whether the results would have been so favourable if an active infection had been set up. Secondly the dose of antitoxin used was large 2,000 units per kilo of monkey. This corresponds in man to 120,000 units administered in a single dose. To give this, using a highly potent serum containing 800 units per ml., would necessitate the injection of 150 ml.—an amount far in excess of that which could be administered intrathecally at one time. The average daily dose given in human practice often by two or three routes—has been from 1,000 to 30,000 units; larger doses have not often been employed. If the quantities suggested by Sherrington's work are really necessary—and experience suggests that smaller doses are inadequate—then it would seem desirable to devote attention to the production of more potent antitoxic sera than those which we now possess.

Firor (1940) obtained similar results to those of Sherrington in dogs given 2 M.L.D. of toxin. Intrathecal antitoxin by the cisternal route saved 51 of 70 dogs (73 per cent.), by the lumbar route 19 of 30 dogs (63 per cent.), whereas intravenous antitoxin saved only 5 of 20 dogs (25 per cent.).

The conclusion from this experimental work (see also Friedemann, Hollander and Tarlov 1941) finds some support in the experience of clinical observers who have succeeded in curing severe tetanus by the use of very large doses of antiserum, sometimes administered by the cisterna magna (see Paterson 1930, O'Carroll 1931, Bell 1931, Nabarro 1932). Nabarro, for example, injected no fewer than 633,000 units of antitoxin within the space of 9 days, while Paterson used nearly 2 million units.

Even more suggestive, however, are the figures reported by Yodh (1932) at the J.J. Hospital in Bombay. Before he took charge, tetanus patients were treated with sedatives, and were given antitoxin intravenously and intramuscularly in doses of about 20,000 units a day. After taking charge, Yodh continued this treatment for a time, using, however, slightly larger doses of sedatives and serum. Later, he increased the dose of antitoxin a little more, and injected part of it by the lumbar intrathecal route. Still dissatisfied with his results, he started giving serum intrathecally by the cisterna magna, as well as intravenously, intramuscularly, and subcutaneously around the wound. Usually only one cisternal injection was made, 20–30 ml. of serum being injected. The total amount of serum administered to any given patient varied from 40,000 to over 400,000 units. The results are given in Table 175.

The cases were not strictly consecutive. Of a total of 229 cases treated by Yodh himself, 15 were not given serum treatment for one reason or another, sometimes because they were moribund. Since the distribution of these cases in point of time is not stated, it is impossible to know how much allowance to make on the figures yielded by the serum-treated patients. The results, however, do suggest very strongly that the intrathecal administration of serum by the cisterna magna route

TABLE 175

EFFECT OF ROUTE AND DOSE OF SERUM INJECTIONS IN HUMAN PATIENTS SUFFERING FROM TETANUS (Yodh 1932).

Mode of Serum Treatment.	No. of Patients.	No. died.	Case mortality per cent.
20,000 units per diem by *im.* and *iv.* routes . .	49	38	77·6
30,000–40,000 units for 1st day, 20,000 units later, by *im.* and *iv.* routes	80	52	65·0
Larger doses of serum by *im.*, *iv.*, and lumbar intrathecal routes	22	14	63·6
Total of 40,000 to 400,000 units by *im.*, *iv.*, *sc.*, and cisterna magna routes	112	53	47·3

im. = intramuscular: *iv.* = intravenous: *sc.* = subcutaneous.

is definitely beneficial. Clinically, some improvement was generally noticeable within 24 hours of the cisterna injection.

From Sherrington's results on monkeys and Yodh's results on human beings, it would appear that intrathecal administration of serum is more beneficial than injection by any other route. Why this should be so, it is difficult to understand. On *a priori* grounds one would expect that antitoxin conveyed by the blood stream would come into more intimate contact with the affected nerve cells than when it was injected into the cerebrospinal fluid. The superiority of the intrathecal route is, however, not yet completely substantiated. Florey and Fildes (1927), for example, obtained no better results in the therapeutic treatment of rabbits by the intrathecal than by the intravenous method. Moreover, Paterson (1930), who treated 26 consecutive children suffering from tetanus with only 7 deaths, used the intravenous and intramuscular routes. It must be noted, however, that Florey and Fildes' experiments were carried out on so few animals that a small advantage possessed by the intrathecal route may quite well have been obscured, while Paterson used

enormous doses of serum, varying from a total of 120,000 to one of 1,970,000 units. Others have strongly deprecated the intrathecal route on the ground that antitoxin may be a severe irritant, and that the ensuing inflammatory reaction may do more harm than the antibody does good (see, for example, Wainwright 1926, Spaeth 1941).

If we accept the view that tetanus toxin is irrevocably fixed to nervous tissue by the time tetanus is manifest, the therapeutic effect of toxin will be limited to the neutralization of toxin in the blood, lymph and tissue fluids, and of toxin as it is absorbed from the local lesion.

The experiments of Abel and others (1938) suggest that, in addition to toxin already fixed by the nerve tissue, some will be adsorbed but not "fixed" to other nerve tissue, and in these circumstances can be neutralized by antitoxin. Therapy should, therefore, be directed to maintaining an effective concentration of antitoxin in both the circulation and the central nervous system. Large doses of antitoxin have been recommended. Aside from their reported success, these have a theoretical justification, for the action of toxin on the capillary endothelium seems relatively slight, and consequently does not induce a ready permeability to circulating antibody. The presence of high concentrations in the blood may ensure that, even with a low degree of capillary permeability, the antitoxin content of extravascular intoxicated tissue may be raised to a therapeutically effective level.

Summarizing, the evidence indicates that in the therapeutic treatment of tetanus large and repeated doses of serum are required. In practice it seems advisable to administer the serum by the intramuscular, intravenous, and intrathecal routes. There is evidence that injection into the cisterna magna is of more benefit than by the lumbar puncture route.

Active Immunization.

Since the demonstration of the antitoxinogenic value of toxoid (formaldehyde-treated toxin; Descombey 1924) and alum-precipitated toxoid (Bergey 1934, Jones and Moss 1936), these agents have been widely tested in man. Owing to the low incidence of tetanus in peace-time, the efficacy of active immunization has been for the most part judged by the antitoxin response in the inoculated subject, and it is only in the last few years that indications of its prophylactic value have been obtained.

There is little doubt that the injection of two to three doses of toxoid at properly spaced intervals raises the antitoxin content of the blood within a few months to the level usually obtained by the injection of a prophylactic dose of antitoxin; and there is good reason to suppose that a given blood level indicates a far higher degree of immunity in the actively, than in the passively immunized person. Thus d'Antona and Valensin (1937) found that actively immunized guinea-pigs with blood levels of 0·012 to 0·05 A.U. per ml. withstood 200 M.L.D. of toxin, while passively immunized animals with 0·2 to 0·5 A.U. per ml. either died or developed severe local tetanus (see also Sneath, Kerslake and Scruby 1937). The difference may in part be due to a better distribution of antitoxin in the actively immunized animal and to the rapid production of antitoxin in an animal conditioned by the previous antigenic stimulus. That the antibody-forming apparatus is conditioned in this manner is clear from the results of injecting toxoid some months after the primary series of injections. Not only is the antitoxin content raised to higher levels than the maxima achieved after the previous injections, but the levels are maintained for longer periods. The response to this secondary, stimulating dose is very striking; the antitoxin content of the blood rises rapidly, and an average titre of over 10 A.U. per ml. is often reached—more than 100 times as high as that following the primary series of inoculations.

In practice, the interval between the primary and secondary doses is usually 3–12 months, but there is some evidence (see Regamey 1944) that the response is even greater after a lapse of 2–3 years. It should be noted that there are great individual variations in response, both to the primary and secondary injections (see Ramon and Zoeller 1933, Sacquepée and Jude 1937, Boyd 1938, Marvell and Parish 1940, Prigge 1940, Evans 1941, 1943, Peshkin 1941, 1944, Fraser *et al.* 1943).

It is administratively convenient to mix toxoid with enteric vaccines. The response to toxoid is reported by some to be enhanced by the presence of vaccine, by others to be unaffected (see, for example, Maclean and Holt 1940, Regamey 1944, and Chapter 50).

Mass immunization is practised mainly in the fighting services. The procedure varies to some extent. Boyd (1938), aiming at a minimum level of 0·01 A.U. per ml., recommends two doses of 1 ml. of toxoid at an interval of 6 weeks, followed by a stimulating dose 9 months later. In the American Army three doses of 1 ml. are given at intervals of three weeks, followed by a fourth dose 1 year later or before military operations (Long 1943).

The efficacy of Boyd's method of immunization was substantiated by the absence of tetanus among immunized soldiers of the British Expeditionary Force in France 1940. Although a large proportion of them failed to receive a prophylactic dose of antitoxin upon wounding no case occurred in over 16,000 actively immunized men, whereas 8 cases occurred in 1,800 men who had not been immunized (Bensted 1940). During the years 1940–42 of the North African campaign, the incidence of tetanus was 0·13 per 1,000 wounded in the actively immunized, 1·6 per 1,000 wounded in the non-immunized (Boyd and MacLennan 1942). Of the 18 patients with tetanus in Boyd and MacLennan's series, only three were known to have received the routine prophylactic dose of antitoxin after wounding; in 5 there was no record, but the remaining 10 had had none. The protection afforded by active immunization was clearly not complete, for 5 cases, 3 of them fatal, occurred in the immunized group. In one of the fatal cases, the evidence suggested a poor antibody response to the toxoid. The possibility of a poor response to primary injections of toxoid emphasizes the need, not only for a secondary stimulating dose during routine immunization, but also for passive immunization on wounding.

It is the practice in the French and American armies to enhance immunity at the time of wounding by a stimulating dose of toxoid. This method has the advantage over serum prophylaxis that toxoid keeps better than antitoxin and is, therefore, simpler to use in the field, and that it does not lay the foundation of a serum-hypersensitiveness. The antibody response to its injection is, however, relatively slow, taking from 2–5 days to develop; and though the average incubation period of tetanus is about 7 days, the more severe infections, in which prophylaxis is of prime importance, develop as a rule more rapidly, before in fact the antitoxin response is manifest. Moreover, aside from a natural insusceptibility to active immunization that characterizes a small proportion of human subjects, the presence of shock, fatigue, cold, and malnutrition in the wounded man may inhibit the antibody response.

Zuger, Greenwald and Gerber (1942) record that immunization with toxoid protected a high proportion of guinea-pigs against a lethal dose of tetanus spores given several months later. When a stimulating dose of toxoid was injected at the time of infection, the protection rate was higher but the active immunity achieved was not solid; some of the guinea-pigs developed local tetanus.

On *a priori* grounds it might be best to give severely wounded patients combined active and passive immunization as soon after receipt of the injury as possible,

so as to ensure protection against rapidly fatal tetanus during the lag phase elapsing between the giving of the secondary stimulating dose of toxoid and the active formation of antitoxin. (For combined immunization with antitoxin and toxoid, see Chapter 50).

The standardization of tetanus toxoid for use in man is based on the antigenic response in guinea-pigs. British regulations for instance require that more than 9 guinea-pigs must be injected with five times the human dose, and that after 6 weeks, at least two-thirds of the guinea-pigs must contain at least 0·1 A.U. per ml. of blood serum.

The Lf value of a toxoid preparation is not a reliable guide to its antigenicity (see, for example, Glenny and Stevens 1938, Lahiri 1940, Koerber 1943, Moloney and Hennessy 1944). On the ground that the antitoxin levels in the serum of an immunized guinea-pig are not a full measure of active immunity, Istrati, Kicksch and Prigge (1940) recommended a protection test, in which the resistance of immunized and normal animals to a standard dose of toxin is compared. Mouse protection tests on these lines have also been devised (see Lahiri 1942, Greenberg, Morrell and Gibbard 1943, Koerber and Mook 1943).

Tetanus in Animals.

The natural disease attacks horses more frequently than other animals, and is especially common in warm and tropical countries. In Northern Europe the incidence is low; thus in the years 1899–1908 in the Prussian Army the annual average of horses developing the disease was only 0·6 per 1,000 (Hutyra and Marek 1912).

Tetanus occurs less frequently in cattle, sheep and goats, though it is not uncommon in ewes after parturition and in lambs after docking; it is uncommon in the pig and the dog.

Infection occurs by contamination of wounds of skin, or of raw surfaces; nails in the hoof, castration, tail docking, harness galling, bone fractures, and imperfect treatment of the navel at birth, seem to be responsible for the majority of cases.

Prophylactic injection of antitoxin is recommended previous to operations on the horse. Nocard (see Mohler and Eichhorn 1911) reported that in 2,727 horses which were given serum after various operations not a single case of tetanus developed, whereas during the same period 259 cases developed in horses that were not inoculated (number not stated). As a rule 500 units is sufficient. The passive immunity so conveyed lasts longer in the horse than in other animals because the serum is homologous, and is therefore excreted more slowly (Mohler and Eichhorn 1911). Buxton and Glenny (1921) were able to render horses actively immune by 3 injections at intervals of 3 days of a toxin-antitoxin mixture. A month later the animals withstood 2,000 guinea-pig M.L.D. of crude toxin. They recommend this method for the immunization of army horses (see also Glenny, Hamp and Stevens 1932).

Therapeutic injection of serum has not been followed by satisfactory results (Mohler and Eichhorn 1911).

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CHAPTER 78

GAS GANGRENE: ANAEROBIC INFECTIONS OF ANIMALS

GAS GANGRENE IN MAN

In the pre-antiseptic days the formation of sloughs, the occurrence of massive necrosis, and the onset of gangrene, so frequently followed upon any extensive operative procedure that they were regarded as more or less inevitable features of surgical practice. With the advent of antiseptic surgery, and the consequent healing of wounds by first intention, these processes became less and less common, till eventually even the names by which they were described took on an archaic air. It was therefore not unnatural that when gas gangrene appeared in the early days of the war of 1914–18, many surgeons were confronted with it for the first time. It was the older surgeons, who had had experience of pre-Listerian methods, who recognized its true nature, and who pointed out the impossibility of preventing it by the modern aseptic methods. Nevertheless, though the disease was old, its study had to be taken up anew. The problems involved were attacked with such success that by the end of the war gas gangrene had ceased to be of more than minor importance. In peace time the disease is uncommon, but is occasionally met with after severe compound fractures, and during the puerperium, particularly when following illegal abortions (see Hill 1936).

In this chapter we can give only an outline of the bacteriological investigation of gas gangrene; for a full description the reader is referred to the monograph of Weinberg and Séguin (1918), and to the Report of the Anaerobic Committee (see Report 1919).

Bacteriology.—In 1914 the incidence of gas gangrene amongst the British Expeditionary Force amounted to over 12 per cent. of the number wounded; of these 20–25 per cent. died. By 1918, owing to earlier evacuation of the wounded, excision of wounds, the employment of an antiseptic technique, and other measures, the incidence had fallen to under 1 per cent. (Report 1919). Indeed, in the Base Hospitals in 1918, amongst a total of about 25,000 wounded reported on by Bowlby (1919), only 84 patients developed serious or massive gas gangrene—an incidence of 0·34 per cent. In the French and German armies the incidence appears to have been rather higher.

Examination of the wound of a man dying of gas gangrene revealed a number of organisms, aerobic and anaerobic; it was uncommon to meet with only one species of bacterium. Thus in an examination of 91 cases, Weinberg and Séguin (1918) found anaerobes in 24, and anaerobes plus aerobes in 67; in only 10 of the former group was a single species isolated—monomicrobial infection. The diversity of flora was so great that no fewer than 38 different combinations of anaerobic bacteria occurred in their 91 cases. To separate these different organisms

and to estimate their particular rôle in the genesis of the disease was a heavy task; the results obtained by the French and English workers, however, agreed sufficiently well for it to be possible to state definitely that certain anaerobes, alone or in combination, were primarily responsible for gas gangrene (Table 176).

TABLE 176

THE FREQUENCY OF DIFFERENT ANAEROBES IN GAS GANGRENE (Report 1919).

	Weinberg and Séguin.	McIntosh.	McIntosh.	Henry.
	91 Cases.	41 Cases.	52 Cases.	50 Cases.
	Per cent.	Per cent.	Per cent.	Per cent.
Cl. welchii (*B. perfringens*)	77	43·9	67·3	80
Cl. œdematiens	34	—	4·0	10
Cl. sporogenes	27	36·5	38·7	—
Cl. fallax	16·5	—	—	6
Cl. septicum	13	19·5	16·3	16

Numerous other anaerobic bacilli were also found, such as *Cl. histolyticum*, *Cl. aerofœtidum*, *Cl. putrificum*, *Cl. bifermentans*, and *Cl. tertium*. Of the aerobes commonly encountered the majority consisted of diplo-streptococci and organisms belonging to the *Proteus* group (Weinberg and Séguin 1918). In 37 of Weinberg and Séguin's cases only one species of anaerobe was isolated; of these 29 were *Cl. welchii*, 5 *Cl. œdematiens*, 1 *Cl. septicum*, 1 *Cl. fallax*, and 1 *Cl. aerofœtidum*.

The suggestion that each of these organisms was capable by itself of giving rise to gas gangrene was borne out by animal experiments. With the possible exception of *Cl. sporogenes*, these five organisms were the only ones that could reproduce in animals a disease simulating the condition in man. With regard to *Cl. sporogenes* it is not clear whether it is pathogenic by itself. The work of the English bacteriologists suggested that it played a subsidiary but important rôle in assisting *Cl. welchii*, *Cl. œdematiens*, and *Cl. septicum* in their attack on the tissues, but that by itself it is harmless. On the other hand, Weinberg and Séguin regarded it as potentially though weakly pathogenic.

From his study of wound infection in the British Army campaigns in North Africa, 1940 to 1942, MacLennan (1943, 1944*a*) recognized three clinical conditions associated with clostridia, namely, simple contamination which occurred at some stage in the history of 20–30 per cent. of all the wounds examined, "anaerobic cellulitis" developing in about 5 per cent. of contaminated wounds, and "clostridial myositis," the true gas gangrene, developing in about 1·5 per cent. of wounds containing anaerobes, and in about 0·34 per cent. of all wounds.

The clostridia most frequent in 259 contaminated wounds were *Cl. tertium* (46·7 per cent.), *Cl. butyricum* (34·3 per cent.), *Cl. welchii* (23·5 per cent.) and *Cl. sporogenes* (20·1 per cent.). Unidentified clostridia were present in 13·2 per cent.; the incidence of *Cl. œdematiens* was 5·8 per cent., and no *Cl. septicum* was found. In anaerobic cellulitis, characterized by foul, sero-purulent infection of the depths and crevices of the wound, without progressive involvement of muscle, the predominant organisms were proteolytic and non-toxigenic clostridia, and *Cl. welchii*. In 146 cases of true gas gangrene, the pathogenic clostridia were as follows: *Cl. welchii* (57 per cent.), *Cl. œdematiens* (37·7 per cent.), *Cl. septicum* (20·0 per cent.), *Cl. tetani* (13·1 per cent.), *Cl. histolyticum* (6·9 per cent.), *Cl. bifermentans* (4·8 per cent.) and *Cl. fallax* (1·4 per cent.). As the fighting moved from desert to more cultivated country, the incidence of gas gangrene was doubled and the

percentage of *Cl. welchii* infection increased. The frequency of the pathogenic clostridia when they were present singly in wounds was as follows: *Cl. welchii* 34·3 per cent., *Cl. œdematiens* 17·1 per cent., *Cl. septicum* 4·8 per cent., *Cl. histolyticum* and *Cl. bifermentans*, each 0·7 per cent. In other cases more than one species was isolated. Anaerobic streptococci (see Chapter 67) were found in 8·8 per cent. of cases.

Summing up, we may say that gas gangrene may be caused by a number of different anaerobic bacteria, usually clostridia, but sometimes by anaerobic streptococci. Usually the clostridia are present either in combination with each other or with aerobic organisms. The three most important anaerobes are *Cl. welchii* (*B. perfringens*), *Cl. œdematiens*, and *Cl. septicum* (*Vibrion septique*); of less importance are *Cl. bifermentans*, *Cl. fallax*, *Cl. histolyticum*, and *Cl. sporogenes*. The presence of pathogenic clostridia in a wound, however, is not necessarily indicative of a gas gangrene infection. Most commonly they appear to be simple contaminants of wounded tissues; they are frequently associated with cellulitis, and only infrequently with true gas gangrene.

Reproduction of Gas Gangrene in Animals.

The experimental disease caused by *Cl. welchii*, *Cl. septicum*, and *Cl. œdematiens* has already been described in Chapter 36.

Each of these organisms, it will be remembered, forms true exotoxins (Bull and Pritchett 1917*a*, *b*, Report 1919, Weinberg and Séguin 1918), which apparently possess hæmolytic, leucocidal, and necrotic powers (Kamen 1904, Eisenberg 1907, Simonds 1915, Weinberg and Séguin 1918, Report 1919, Weinberg and Ginsbourg 1927). The most potent toxin is formed by *Cl. œdematiens*; indeed the disease associated with this organism partakes more of an intoxication than a true infection.

Mode of Development of Gas Gangrene in Man.

We have already seen that the injection of a broth culture of *Cl. welchii* or *Cl. septicum* into a guinea-pig will give rise to a disease simulating gas gangrene. If, instead of using a whole broth culture, we wash the organisms thoroughly to free them from toxin, no disease is set up. Likewise, saline suspensions washed off an agar slope are non-pathogenic. Large numbers of the bacilli can be injected without giving rise even to a local lesion. But if to the toxin-free bacilli a sub-lethal dose of toxin (broth filtrate) is added, the mixture proves intensely virulent. A protocol from De Kruif and Bollman's paper (1917) will illustrate this point:

Effect of intramuscular injection into guinea-pigs of—

1·0 ml. *Cl. welchii* broth filtrate	Survived.
0·05 ml. washed organisms + 0·5 ml. sterile broth . .	Survived.
0·1 ml. „ „ + 0·5 ml. „ „ . .	Died 36 hrs.
0·000,01 ml. „ „ + 0·5 ml. broth filtrate . .	Died 19 hrs.

It will be seen that a non-lethal dose of filtrate decreased the M.L.D. of the washed bacilli several thousand times.

The broth filtrate contains an aggressin which enables the bacilli to proliferate in the tissues. Without this the bacilli are partly lysed by the tissue fluids and partly taken up by phagocytes; with it neither lysis nor phagocytosis occurs, but the organisms divide, produce fresh toxin, and bring about the death of the

animal. The aggressin is probably the α-toxin, for its action can be completely inhibited by specific antitoxin (see Stewart 1943*a*). Of the toxic components in culture-filtrates of *Cl. welchii* Type A, antibodies to the α- and θ-toxins and to the hyaluronidase (see Chapter 41) are present in preparations of antitoxin, and of these, only the antitoxin has protective properties (Evans 1943*a*, *b*). In other words, the bacilli apart from their toxin are powerless to overcome the resistance of the tissues. How the broth filtrate acts is not clear, but since both *Cl. welchii* and *Cl. septicum* are known to bring about in culture a very steep fall in Eh, it is possible that the filtrate contains reducing substances capable of establishing a sufficiently low O-R potential to enable the spores to germinate; or that the tissues themselves develop a low O-R potential as a result of intoxication. Once germination has started, the organisms probably produce their own reducing system, which enables them to multiply even in the presence of healthy tissue.

Toxin is not the only substance, however, that can break down this resistance. Bullock (Gye) and Cramer (1919), whose work has been described in the last chapter, found that small quantities of ionizable calcium salts—notably calcium chloride—exerted a similar kataphylactic action. Colloidal silicic acid behaved in the same manner.

Since the organisms introduced into a wound are devoid of toxin, being frequently in the sporing stage, their germination must depend on other factors. In the opinion of Bullock and Cramer the determining factor is some kataphylactic substance that is carried into the wound with them. Earth containing calcium salts or silicic acid is suggested as the probable accessory agent. The work of Fildes (1927) and Russell (1927) renders it highly probable that such substances as earth or calcium chloride act by causing foci of necrosis, in which the oxidation-reduction potential becomes sufficiently reduced to enable the anaerobic bacterial spores to germinate (see Chapter 77). Fragments of clothes in the wound also appear to act as foci for the germination of spores. The observation of Fildes (1935) that in fluid media cellulose fibres induce an O-R potential low enough to permit the germination of tetanus spores is clearly relevant in this connection.

The possible importance of secondary infection is difficult to assess; it may well be that highly proteolytic organisms such as *Cl. histolyticum*, *Cl. putrificum*, *Cl. bifermentans*, and *Proteus vulgaris* assist in preparing the way for the gas-gangrene bacteria, not only by destroying the tissues, but by lowering the O-R potential to the critical level for germination (see Hewitt 1936). Bacteria like certain staphylococci and streptococci, as McClean and Rogers (1944) suggest, may promote the spread of the gas-gangrene organisms by releasing hyaluronidase into the tissues of the wound. Similarly mechanical injury, crushing of the tissues, effusion of blood, and fracture of bone may have the same effect. But of the precise effect of each of these agents we are entirely ignorant.

That some determining factor is necessary, however, is shown not only experimentally in animals, but by the observation in man that *Cl. welchii*, *Cl. septicum* and *Cl. œdematiens* may all be present in a wound without setting up gas gangrene.

Working with *Cl. welchii* Robertson and Keppie (1941), Keppie and Robertson (1944) found that the *in vitro* α-toxigenic capacity was in general highest in gas-gangrene strains, less in those from cases of "anaerobic infections," and least in contaminating wound strains. There were, nevertheless, marked exceptions, and they considered that almost all the strains studied were capable of inciting gas gangrene in suitable circumstances. The toxins of *Cl. welchii* have also been credited with other specific actions, especially

upon the heart (see Nicholson 1934–5, Kellaway, Trethewie and Turner 1940), but the relation of results obtained with "acute" physiological experiments to the syndrome observed in human gas gangrene is not clear. Nor is it known whether the α-toxin owes its toxicity to its lecithinase activity (see Chapter 36), though it is clearly possible that the disruption of lipo-protein complexes in the body may have far-reaching effects on the metabolism of the cells containing them.

"The disease begins, not when a wound has become infected with the pathogenic anaerobes, but from the moment when a group of these bacteria have been enabled to surround themselves with a toxin sufficiently concentrated to abolish the local defences of the tissues" (Report 1919). Once this has occurred, the organisms multiply, produce more toxin, and rapidly invade the tissues. Aside from their aggressive action in the infected tissues, we are largely ignorant of the mode of action of the various clostridial toxins in man. The toxin of *Cl. welchii* has a deleterious action on the suprarenals, and it is partly owing to the consequent lack of adrenalin in the blood that exhaustion and death occur so quickly. This paralysis of the suprarenals is apparently aided by hæmorrhage, exposure to cold, and absorption of the substances formed by growth of the bacilli in the tissues.

Diagnosis.

It cannot be too strongly emphasized that the diagnosis of gas gangrene must primarily be made upon clinical grounds. As the figures on p. 1771 show, the demonstration of one of the pathogenic clostridia in a wound, and especially of *Cl. welchii*, is by itself of little pathognomonic significance. The technique of anaerobic bacteriology is at present too laborious and time-consuming to permit much in the way of confirmatory evidence that will be of value in a disease whose incubation period may be as short as 4 hours, and is on the average 1–2 days. Direct examination, however, of the wound material may yield valuable information, especially for the diagnosis of streptococcal myositis, and should never be omitted (see Memorandum 1943, Hayward 1945).

McClean and his colleagues (McClean, Rogers and Williams 1943, McClean and Rogers 1943, 1944) noted the production of large quantities of hyaluronidase in the gangrenous lesions of guinea-pigs infected with *Cl. welchii*, *Cl. septicum* and with some strains of *Cl. œdematiens*, and devised a simple technique for its rapid recognition in wound exudates, the various hyaluronidases being identified by inhibition with specific anti-hyaluronidase sera. Owing to the frequent presence of hyaluronidase-producing *Str. pyogenes* and *Staph. aureus* in wounds, antisera against the enzymes of these organisms were included in the test. Using this method, MacLennan (1944*b*) obtained only 3 positive results in 12 cases of proved gas gangrene of man—a result which has been attributed to destruction of hyaluronidase by other bacteria in the wound, or its neutralization by mixed antitoxin (see below) given prophylactically to the wounded soldier. The method is noteworthy as providing a possible means of making a presumptive bacteriological diagnosis within an hour of examining a wound.

A full bacteriological examination of a wound should include direct plating, and seeding into media containing cooked meat, thioglycollic acid (Brewer 1940) or other reducing substances that promote anaerobic conditions. The cooked meat culture is plated at intervals to detect the scanty or slow-growing bacilli. The tendency of clostridia to form spreading colonies on the surface of solid media may be counteracted by the use of nutrient gels containing up to 8 per cent. of agar devised by Hayward (Miles and Hayward 1943), which permit the ready isolation of pure cultures. After incubation fluid cultures may

be heated to 80° C. for 20 minutes to destroy non-sporing forms before seeding on to plates. By greater degrees of heating, species with more resistant spores, like *Cl. œdematiens*, may often be obtained in pure culture. *Cl. welchii* and *Cl. bifermentans* may be readily recognized by the Nagler reaction (see Chapter 34) in primary plate culture on a medium containing 20 per cent. human serum. Lecithinase activity of these two organisms produces an opacity in the medium, but none in similar medium containing *Cl. welchii* antitoxin (Hayward 1943).

Pathogenic may be separated from non-pathogenic organisms by animal inoculation. Moreover the individual species of pathogenic bacteria may be separated by injection into protected animals. Thus, if a mixture of *Cl. welchii*, *Cl. septicum*, and *Cl. œdematiens* is inoculated into a guinea-pig that has received a dose of antiserum prepared against the latter two organisms, then *Cl. welchii* alone will proliferate and invade the blood stream. In the absence of laboratory animals, serologically controlled tests of specific hæmolysin production may be used to identify *Cl. welchii*, *Cl. septicum* and *Cl. œdematiens* (Hayward 1945). For a full description of the methods of isolation and purification of the anaerobes the reader is referred to Report (1919) and Hayward (1945).

Blood cultures during life are frequently positive (Weinberg and Séguin 1918). *Cl. welchii* and *Cl. septicum* are especially liable to invade the blood; *Cl. œdematiens*, which acts chiefly by virtue of its toxin, often remains confined to the local lesion.

Prophylaxis and Treatment.

Passive Immunization.—Towards the end of the war of 1914–18, antitoxic and antibacterial sera were prepared against *Cl. welchii*, *Cl. œdematiens* and *Cl. septicum*. No extensive trial was possible, but the results of prophylaxis in a small series of badly wounded soldiers, and of treatment in a number of soldiers suffering from gas gangrene, with mixtures of these sera suggested that both procedures were beneficial (Weinberg and Séguin 1918, Report 1919).

In experimental gas gangrene, both antitoxins and antibacterial sera containing O antibody are protective. But since none of the chief gas-gangrene clostridia is antigenically homogeneous, the efficacy of an antibacterial serum is confined to infections with a given serological type (see Robertson and Felix 1930, Henderson 1934, 1935). It is clearly impracticable to maintain antibacterial sera specific for the multiplicity of serological types. On the other hand the toxic antigens in culture filtrates of these clostridia, though they vary in the content of different components, are to a large extent species-specific, and for routine therapy a mixture of antitoxins can therefore be used.

Several different methods have been employed in the *standardization* of antiserum against gas gangrene (see Dalling *et al.* 1928, Buttle and Trevan 1928, Glenny, Llewellyn-Jones, and Mason 1931, Weinberg, Davesne, and Prévot 1932, Bengtson 1934, Hartley and White 1935, Walbum and Reymann 1935, Ssilanowa 1935, Weinberg and Guillaumie 1938, Ipsen 1939, Ramon 1940, Guillaumie 1941, 1942, 1943, Nagler 1941, Seal and Stewart 1941). The two chief methods used for titrating the antitoxin content are intramuscular or intravenous inoculation of mice, and intracutaneous inoculation of guinea-pigs or rabbits, with toxin-antitoxin mixtures. Other methods include flocculation tests analogous to the Ramon titration of diphtheria antitoxin, and, with *Cl. welchii*, neutralization of the lecithinase activity of the α-toxin. The *in vivo* titrations are at present the most reliable, and will probably remain so until the significance of the various toxic components in culture filtrates is fully understood. The international standards laid down by the League of Nations may be briefly defined.

as adjuvants to surgery, and in combination with antitoxin (see MacLennan 1943). Jeffrey and Thomson (1944) record a striking arrest of clostridial myositis by penicillin, but note that in some cases the arrest was followed by a fatal intoxication, apparently due to absorption of substances from the retained and degenerating muscle.

Active Immunization.—Formol and alum-precipitated toxoids induce in guinea-pigs and rabbits protective antitoxic immunity to experimental infection with *Cl. welchii*, *Cl. œdematiens* and *Cl. septicum* (Penfold and Tolhurst 1937, Penfold *et al.* 1941, Kesterman and Vogt 1940, Kolmer 1942, Stewart 1942, Robertson and Keppie 1943). As with other toxoids, a stimulating dose some time after the primary injections is necessary for high degrees of immunity. In man, the antitoxic response to these toxoids is good (Penfold and Tolhurst 1938, Stewart 1943*b*); but at present there are no data from which the value of prophylactic immunization can be estimated. It should be noted that in the guinea-pig, the response to a stimulating dose was not demonstrable for 3–10 days (Robertson and Keppie 1943). Immunization of man by means of a stimulating dose at the time of wounding is not, therefore, likely to affect the outcome of a disease of which the incubation period is commonly less than 2 days.

Other Clostridial Infections.—The active participation of toxigenic clostridia, especially of *Cl. welchii*, in the severe and sometimes fatal "toxæmia" that accompanies acute obstruction of the intestine or acute appendicitis has long been a matter of debate. Williams (1926) obtained evidence suggesting that the toxæmia was due to the formation of *Cl. welchii* toxin in the lumen of the obstructed small intestine, and that antitoxin was beneficial in the treatment of such cases. Paralytic ileus following acute inflammation in the abdominal cavity appeared to have the same effect as mechanical obstruction in the genesis of toxæmia; clostridial infection of the inflamed tissue was not observed. Weinberg (Weinberg *et al.* 1928*b*, Weinberg 1929), however, observed that clostridia could be obtained from 30 per cent. of acute lesions of the appendix (subsequent observers have recorded higher figures); and he tested the effect of antisera prepared against the gas-gangrene organisms in the treatment of conditions associated with an anaerobic invasion of the tissues (Weinberg and Howard 1927, Weinberg *et al.* 1928*a*). At most, it can be said that such antisera appear to reduce the case-fatality rates, especially when used in conjunction with surgical treatment (for later references see Priestly and McCormack 1936).

Bower, Burns and Mengle (1938) induced a fatal peritonitis in dogs by ligation of the appendiceal vessels, followed by a purgative dose of castor oil; *Cl. welchii* antitoxin reduced the mortality, and prolonged the lives of animals that eventually succumbed to the infection. Normal horse serum was also protective, but to a lesser extent—an effect attributed to the natural antitoxins in the serum. Prophylactic immunization with *Cl. welchii* toxin also reduced the death rate from this experimental disease (Mengle *et al.* 1938). The active participation of *Cl. welchii* may also be inferred from the fact that an antitoxin response is demonstrable in patients with appendicitis. Thus, Bower, Mengle and Paxson (1938) found that 22·2 per cent. of patients with acute appendicitis, 46·6 per cent. of those with active or quiescent pelvic peritonitis, and 69·0 per cent. of those with septic peritonitis after perforation, had more than 1 unit of *Cl. welchii* antitoxin per ml. of circulating blood. The corresponding figure in control patients was 0·0 per cent.

ANAEROBIC INFECTIONS OF ANIMALS

Blackleg and Gas Gangrene

SYNONYMS: Symptomatic anthrax, quarter-evil, blackquarter.
French: *Charbon symptomatique.* German: *Rauschbrand.*

Blackleg is a disease attacking cattle, sheep, and less often swine (Meyer 1915). It is characterized by the appearance of a crepitant, fluctuating swelling, generally on one of the quarters, followed rapidly by death. Calves are resistant for the first 6 months of life; they then become susceptible and remain so till about 2 years of age. The disease is commoner in the summer than in the winter. It may follow shearing, docking, parturition, or castration in sheep, but more often appears spontaneously. Arloing, Cornevin and Thomas (1881) first showed that the disease could be transmitted to cattle by inoculation of infective material. From the lesions, they isolated an anaerobic spore-bearing bacillus—*Cl. chauvœi.* The washed bacilli are non-infective, but they can be activated by a small dose of broth filtrate, or by substances such as lactic acid, potassium chloride, or sand (Nocard and Roux 1887, Roux 1888, Leclainche and Vallée 1900).

The term *gas gangrene* or *malignant œdema* is sometimes reserved for anaerobic infections of the muscle consequent on trauma. The essential pathology of blackleg and gas gangrene appears to be the same, and the distinction between the terms seems of doubtful value.

Blackleg in cattle seems to be generally caused by *Cl. chauvœi*, and in sheep by *Cl. septicum* (Heller 1920, Gaiger 1924, Weinberg and Mihailesco 1929), though *Cl. chauvœi* infections are not uncommonly found—especially if a bacteriological examination is made shortly after the death of the animal. Albiston (1937) records an outbreak of *Cl. chauvœi* blackleg in sheep, in which the local predisposing factor appeared to be a vaccine injected for immunization against enterotoxæmia (see below).

Vaccination has been practised for many years and has been attended by a considerable amount of success. Arloing, Cornevin and Thomas used two vaccines of different virulence; the first was prepared by exposing the dried juice from a blackleg tumour to 100–104° C. for 6 hours; the second by exposing it to 85–90° C. for the same time. Inoculation of cattle was practised subcutaneously on the under surface of the tail. Statistics compiled by Cornevin (1895) are given in Table 177.

TABLE 177

RESULTS OF VACCINATION AGAINST BLACKLEG IN CATTLE.

	No. of Animals Vaccinated.	Mortality due to vaccination.	
		Total.	Per 1,000.
1893	6,696	5	0·75
1894	8,222	8	0·97
1895 first 6 months . . .	6,936	5	0·72

The mortality in the vaccinated animals varied in different districts from 0–10 per 1,000; amongst the unvaccinated animals it varied from 20 to 250 per 1,000.

Several other vaccines have been employed (Roux 1888, Nitta 1919, Dalling 1925. See also *J. comp. Path.* 1895, **8**, 166). A formolized broth culture of *Cl. chauvœi* has been recommended (Leclainche and Vallée 1925, McEwen 1926); under experimental conditions, sheep injected with this vaccine appeared to derive a greater degree of immunity against subsequent inoculation with a living culture than sheep vaccinated with a non-formolized broth filtrate. Henderson's (1932) work suggests that the heat-stable O antigen is likely to prove a powerful immunizing agent. A high degree of protection against intramuscular injection of living cultures is produced in sheep by the subcutaneous inoculation of solutions of the heat-stable antigen (Mason 1936). Antitoxins are less effective against the bacterial invasion. However, sera for therapeutic use have been prepared against *Cl. chauvœi* and *Cl. septicum*, and their intravenous administration in cases of the fully developed disease appears at times to have given favourable results.

Braxy

SYNONYMS: *Bradsot* = quick plague.

Braxy is the term used for a disease of sheep occurring mainly in the north-western part of Europe. Recent work has rendered it clear that more than one type of disease is called by this name. For descriptive purposes we propose to reserve the term braxy for the type of disease characterized by inflammation of the fourth stomach, and to refer to the so-called German braxy by the term black disease or infectious necrotic hepatitis.

The classical or northern braxy occurs on the western coast of Norway, in Iceland, the Faroe islands, and Scotland. Sheep are most susceptible during their first year. The disease breaks out in the late autumn and early winter months when the animals descend from the hills to the pastures of the lowlands. In Scotland the losses from braxy in some districts may amount to as much as 35 per cent. of the first year's sheep (Gaiger 1924). Clinically, in the usual acute form of the disease, death occurs before any symptoms have been noticed; in the subacute form there is weakness, followed by coma, dyspnœa, and death. The duration of the disease is a matter of a few hours. Post mortem, there is severe inflammation of the fourth stomach and duodenum, with œdema and hæmorrhage—sometimes necrosis—of the mucosa and submucous tissue. The peritoneal fluid is often turbid and in excess; there are degenerative changes in the visceral organs, and an acute parenchymatous nephritis.

The causative organism was described by Nielsen in 1888, who found it in large numbers in the stomach wall. It was identified by Gaiger (1922) with *Cl. septicum*. The mode of infection is obscure; experimental feeding with pure cultures of the bacillus apparently fails to reproduce the disease; Jensen was successful only 5 times in 1,545 attempts (Gaiger 1924). Subcutaneous injection produces black-leg; post mortem, lesions may be present in the abomasum simulating those of the natural disease (Hamilton 1902, 1906); there is no evidence however that natural infection occurs by this route. Again we are forced to conclude that some accessory factor must be present before the bacillus is able to invade the tissues. Experimental work on guinea-pigs by Borthwick (1934) suggests that intestinal stasis and exposure of the animal to cold are important factors in determining the invasion of the tissues by *Cl. septicum*. While the feeding of cultures to normal guinea-pigs rarely set up infection, inhibition of peristalsis by narcotine, or pre-

liminary exposure to cold, resulted in the development of a fatal infection in a fairly high proportion of the animals.

A considerable degree of protection is afforded by the use of Jensen's sero-vaccine.

Gaiger (1922) obtained promising results by the use of a sterile filtrate of a culture of *Cl. septicum*. Of 10,340 hoggs (1st year sheep) vaccinated, 2·39 per cent. died of braxy, while among 3,800 control animals the mortality from braxy was 9 per cent. (see Gordon 1934*a*).

Dalling (1925, Dalling *et al.* 1925, 1926) advocated a combined vaccine against blackleg and braxy in sheep. He used a culture filtrate of *Cl. chauvœi* and a mixture of *Cl. septicum* toxin and antitoxin. During the winter of 1924, 549 sheep were inoculated, with a braxy mortality of 0·36 per cent.; of 464 unvaccinated sheep on the same farms and under the same conditions 15·5 per cent. died of braxy. Satisfactory results were also obtained by Gordon (1934*a*) using a formolized whole culture of *Cl. septicum*. Preliminary experiments on sheep showed this to be superior to Gaiger's filtrate vaccine, and it was therefore used for further trial. Of 3,588 vaccinated hoggs, 73 (0·8 per cent.) died of braxy, while of 1,886 control animals on the same farms, 158 (8·4 per cent.) died of this disease. The vaccine is prepared by adding 0·5 per cent. formol to a 15-hour horse flesh broth culture and incubating at 37° C. for 2 days. By this time it should be bacteriologically sterile, and should prove non-toxic when inoculated intravenously into mice in a dose of 0·5 ml. Dungal (1932) in Iceland also reported favourable results from the use of a formolized whole culture vaccine of *Cl. septicum*. Comparison with Jensen's sero-vaccine yielded the following figures. Of 11,308 sheep given the sero-vaccine, the mortality from all causes was 2·44 per cent. whereas of 59,736 sheep given the formolized vaccine, it was only 0·84 per cent.

Black Disease

SYNONYMS: Infectious necrotic hepatitis; German braxy.

This disease occurs in Australia, particularly Victoria, New South Wales, and Tasmania, and in certain parts of Germany, where it is termed braxy. In Germany two types are recognized: (1) Meadow braxy, affecting mainly ewes at pasture in well-watered valleys; and (2) Stall braxy, affecting chiefly young castrated lambs that are being fattened for market (Miessner, Meyn, and Schoop 1931). In Australia the meadow type alone seems to be common. Well-nourished animals are affected. Clinically the course in meadow braxy is usually very acute, and the animals are often found dead before suspicion of illness has been aroused, but in stall braxy symptoms may be present for 12 hours before death. Examination of fresh carcasses reveals the presence of subcutaneous venous engorgement, which gives the skin a dark colour—hence the term black disease—amber-coloured fluid in the pericardial and peritoneal cavities, and characteristic necrotic areas in the liver. Unlike true braxy the abomasum is slightly or not at all affected, and no obvious degenerative changes are present in the visceral organs. The liver lesions are most typical of the disease. Greenish bile-stained areas of necrosis, about 1 cm. in diameter, containing cavities filled with blood clot and immature liver flukes, are usually seen in small numbers. In addition to these non-specific foci, there are a few characteristic, irregularly circular, necrotic areas, 2–3 cm. in dia-

meter, sharply defined, yellowish-white in colour, and surrounded by a wide zone of venous congestion. Bacteriologically the liver can be shown to contain large anaerobic bacilli, which are refered to by the Germans as *Cl. gigas*, but which appear to be identical with, or a variety of, *Cl. œdematiens*.

The pathogenesis of the disease has been elucidated by Dodd (1918, 1921), Albiston (1927), and particularly Turner (1930), who sought for predisposing factors which, as Fildes had demonstrated in the case of infections with *Cl. tetani* (Chapter 77), would increase the reducing intensity of the tissues so as to favour the germination of the spores of *Cl. œdematiens*. By a careful series of observations and experiments, Turner was able to show that in black disease the necessary conditions for growth of the causative organism were found in the necrotic areas produced by invasion of the liver with wandering immature liver flukes of the species *Fasciola hepatica*. In these areas the organisms multiplied, and gave rise to a toxin which was responsible for the main features of the disease. Many sheep were found to be latently infected with the spores of *Cl. œdematiens*, but not until suitable necrotic foci were established by invasion with liver flukes did the infection become active.

Consequent on this work Turner prepared a formolized broth vaccine with which very satisfactory results were obtained in field trials. The figures showed that when two doses were given, the mortality from black disease was reduced on an average by about half, as compared with control animals on the same farm. Even more favourable results attended the use of three doses of vaccine.

Vaccination of over 270,000 sheep in Victoria, Australia, during the years 1933–36 was accompanied by a reduction in the annual mortality due to black disease from 4–15 per cent. to 0·02–0·05 per cent. (Wardle 1936; see also Rose 1936). According to Oxer (1937) alum-precipitated vaccine is a more efficient immunizing antigen.

Osteomyelitis of Buffaloes.—The buffaloes of the Dutch East Indies suffer from a non-fatal osteomyelitis, which particularly affects the humerus and femur. The causative organism is indistinguishable from *Cl. gigas*, except that it is non-pathogenic to laboratory animals. The pathogenesis of the disease is obscure; and inoculation of the buffalo with the organism is not followed by osteomyelitis (Kraneveld 1930, Kraneveld and Djaenoedin 1933).

Infections due to Cl. welchii : Lamb dysentery ; "Struck" ; infectious enterotoxæmia ; pulpy kidney disease

A number of acute toxæmic diseases have been described in different parts of the world, mostly affecting sheep, in which the lesions appear to result from an intestinal infection with a variety of *Cl. welchii*. The pathogenesis of these diseases is to a large extent obscure, but considerable progress has been made in their control by vaccination and serotherapy.

Lamb dysentery is a disease that takes a heavy toll of life among the lambs during their first 2 weeks of life (Gaiger and Dalling 1921). It is particularly prevalent in the border counties of England and Scotland. Pathologically it is characterized by an enteritis varying from a mild congestion of the intestinal mucosa to a condition in which extensive tracts of the small and large intestine become necrosed and ulcerated. On the surface of the inflamed mucosa and ulcerated areas a bacillus is found closely allied to *Cl. welchii*, but differing from it in certain cultural particulars and in its production of a more powerful toxin (Dalling *et al.*

1925, Dalling 1928*b*, 1931–32). This organism is sometimes referred to as the lamb dysentery bacillus, and sometimes as the *agni* variety of *Cl. welchii*. It is at present probably better to call it *Cl. welchii* Type B (see Chapter 36). The organisms are confined mainly to the intestine, and the body tissues are not usually invaded. Dysentery associated with *Cl. welchii* Type B has also been reported in foals (Mason and Robinson 1938).

Protection against the disease can be afforded in one of two ways: (1) the pregnant ewe may be immunized with an over-neutralized toxin-antitoxin mixture, or with a simple formolized culture. Both of these methods of inoculation appear to give satisfactory results. Thus in one field experiment, the mortality from lamb dysentery amongst 741 lambs born of ewes inoculated with the toxin-antitoxin mixture was 1·07 per cent.; amongst 1,157 lambs born of ewes inoculated with a formolized culture it was 1·12 per cent.; while amongst 2,530 control lambs born of uninoculated ewes on the same farms it was 13·09 per cent. (Dalling 1928*b*. For further records see Dalling 1926, Dalling, Mason, and Gordon 1928, 1929). The antibodies generated in the ewe by this method are transferred to the new-born lamb by means of the colostrum (Dalling 1928*a*, Mason, Dalling, and Gordon 1930). (2) The new-born lamb may be injected directly with antitoxin. This method likewise gives satisfactory results. Thus on 15 farms in 1927 the mortality from lamb dysentery amongst 1,122 lambs injected with serum was 0·44 per cent., while amongst 1,241 control lambs on the same farms it was 17·16 per cent. (Dalling 1928*b*).

"Struck" is the local name given to a disease of sheep occurring during the late winter and spring months on the Romney Marsh in Kent. Clinically the symptoms are of short duration, and the case mortality is high. In fresh carcasses the main lesions are those of an acute enteritis and peritonitis, but in animals that have been dead for some hours the subcutaneous and muscular tissues present a picture similar to that found in gas gangrene. The work of McEwen (1930) and McEwen and Roberts (1931) seems to show that the disease is essentially a toxæmia consequent on the multiplication in the intestinal canal, and perhaps the body tissues, of an organism resembling *Cl. welchii*. The name *Cl. paludis* has been suggested for it, but until we know more of the relationship between this organism and other closely allied members of the *welchii* type it is probably better to refer to it as *Cl. welchii* Type C. Vaccination with toxoid is apparently of value in protecting against the disease (McEwen 1935).

Infectious enterotoxæmia is a disease of sheep described by Bennetts (1932) in Western Australia, and apparently due to the absorption of toxin from the small intestine. The organism, for which the name *Cl. ovitoxicum* has been suggested, appears to be more or less identical with Wilsdon's (1931, 1933) *Cl. welchii* Type D, and is better referred to by this name. The same organism appears to be responsible for the condition known as **pulpy kidney disease of lambs,** which has been studied in New Zealand by Gill (1932) and in Wales by Montgomerie and Dalling (1933) and Montgomerie and Rowlands (1933, 1934). Immunization of sheep with a formol-treated culture, which presumably contains both bacterial and exotoxin antigens, appears to lower the incidence of infectious enterotoxæmia (Bennetts 1936, Rose 1936). Death from pulpy kidney is commonest in 3–6-week-old lambs, so that active immunization of the new-born lamb is unlikely to be successful. Protection is achieved by the vaccination of the pregnant ewe, the lambs rapidly acquiring a passive immunity by ingestion of the maternal antitoxin

present in the colostrum (Oxer 1936, Dayus 1938). Gordon (1934*b*), who examined filtrates of intestinal contents from lambs, sheep, and horses that had apparently died of acute toxæmia, was able to demonstrate the presence of a toxin neutralizable by an antitoxin to *Cl. welchii* Type D. His work suggests that this organism may play a part in a number of diseases of sheep, lambs, and perhaps horses.

The conditions governing the formation of a toxin in most of the diseases that we have been discussing are still obscure, and there is a considerable field open for an investigation of the type so successfully undertaken by Turner (1930) in black disease. It is interesting to reflect that whereas anaerobic infections in man and the carnivora are generally preceded by trauma, in herbivora they often occur spontaneously (Heller 1920).

The genesis of enterotoxæmia affords an illustration of the complexity of the problem. The main clinical and pathological findings in this disease appear to be the result of intoxication by the ε-toxin (Nicholson 1934–35, Kellaway, Trethewie and Turner 1940, Gordon *et al.* 1940). In 1935 Bosworth and Glover observed that the lethal power of the toxin of *Cl. welchii* Type D, but not of Types A, B or C, was increased when added to an intestinal filtrate of a normal sheep, guinea-pig or rabbit. But in spite of the increase, exactly the same amount of antitoxin was required to neutralize the toxin, whether it was free or mixed with the intestinal filtrate. These workers suggested that the toxin might form a highly toxic complex with the intestinal tryptic ferments, possessing some of the properties of histamine. More recently Turner and Rodwell (1943) have reported that all kinds of proteolytic enzymes, including those of *Cl. welchii* itself, can "activate" the ε-toxin; they postulate an ε-prototoxin which may be converted by any of these enzymes into ε-toxin. As regards the conditions for toxin formation in the gut, Roberts (1938) concluded from *in vitro* and *in vivo* experiments that in milk-fed lambs, the acidity of the abomasum contents normally prevented any proliferation of *Cl. welchii*. If infective material is present after the ingestion of an abnormally large feed, the acidity is depressed by the casein in the feed, and *Cl. welchii* Type D may proliferate, producing a toxin which is activated in the small intestine.

The source of infection is presumably *Cl. welchii* Type D in the soil, though it is found there only occasionally, Type A or non-toxic *Cl. welchii* being the predominating varieties (see Taylor and Gordon 1940). However, it is known that Type D strains readily lose their power to produce ε-toxin on subculture (Borthwick 1937), and it is possible that many of the Type A strains found in soils and the intestinal content of animals may be degraded Type D strains.

(For an account of the anaerobic toxæmias of animals, see Woodruff 1936.)

For **botulism** in animals see Chapter 72.

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CHAPTER 79

MISCELLANEOUS DISEASES

NECROBACILLOSIS, OZÆNA, RHINOSCLEROMA, GRANULOMA VENEREUM, SOFT CHANCRE, GLANDULAR FEVER, INFECTIVE HEPATITIS, BARTONELLA INFECTIONS, AND VARIOUS OTHER DISEASES

NECROBACILLOSIS

THIS term may be used to cover a wide variety of lesions in man and animals due to infection with the non-sporing anaerobic bacillus known as *Fusiformis necrophorus* (see Chapter 18).

In *man*, infections by organisms of the *F. necrophorus* group, which includes those strains named *B. funduliformis*, seem to be rare. This may result from failure to submit all material from purulent infections to routine anaerobic cultivation. Holicky (1940), for example, records that Gram-negative, anaerobic non-sporing rods and fusiform bacilli were obtained, sometimes in pure culture, from about 2 per cent. of over 5,000 specimens from infected patients in a surgical clinic. Judging from the species-frequency observed in human infections, it is probable that the majority of the strains isolated were of *F. necrophorus* type.

The disease may take the form of purulent or gangrenous inflammation of the skin (see Shaw 1933). The mode of infection is not always clear, but a number of cases have followed bites or injuries from the teeth of animals. More commonly it occurs as a deep-seated infection, with purulent abscesses in the abdominal cavity, liver or lung, many of which appear to be the result of a pyæmia. It often complicates operations on appendix abscesses or other septic lesions of the lower gut. The case mortality among patients with well-established infection is over 50 per cent. The infecting organism is presumably derived from the alimentary tract, though *F. necrophorus* has seldom been isolated from normal stools. In some instances, however, *Fusiformis* strains from infective lesions in man resemble those found in normal tissues. Thus, Spaulding and Rettger (1937) demonstrated cultural and serological similarities between strains from the normal and from the infected mouth and vagina, and from lung abscesses. What relation the disease has to Vincent's angina (see Chapter 80), and to the various necrotic stomatitis conditions in which the closely related organism *Fusiformis fusiformis* seems to play a part, is not clear.

It is impracticable to review the types of human infection that have been reported, but we may note the following. Puerperal sepsis (Harris and Brown 1927, Delbove and Reynes 1941); abdominal, hepatic or lung abscesses (Cunningham 1930, Cohen 1932, Thompson and Beaver 1931, Shaw and Bigger 1934, Beaver, Henthorne and Macy 1934, Henthorne, Thompson and Beaver 1936, Lemierre 1936, Dixon and Deuterman 1937,

Brunner 1937, Buhler, Seely and Dixon 1942); Chandler and Breaks (1941) record a case of femoral osteomyelitis, Forbes and Goligher (1944) a generalized pyæmia following a war-wound infection.

Among other fusiform bacilli *F. fragilis* produces infections in man similar to those of *F. necrophorus* (Thompson and Beaver 1931, Henthorne, Thompson and Beaver 1936). *F. ramosus* (Lemierre, Reilly and Bloch-Michel 1937) and *F. melaninogenicus* (Oliver and Wherry 1921, Burdon 1928, Weiss 1943) are associated with less severe infections, apparently originating, like *F. necrophorus* infections, from the respiratory or genital tract; and though they are sometimes found in the blood stream their power to initiate infection in healthy tissue appears to be limited.

Dack and his colleagues have studied the relation of *F. necrophorus* to ulcerative colitis. They find that the flora of the ulcerated colon differs from that of the normal colon in consisting of anaerobic organisms, with *F. necrophorus* predominating during periods of exacerbation of the disease, and disappearing during periods of quiescence. The organism is not found in the normal colon. Its causal rôle in the disease is not clear, but material from human lesions is said to induce necrotic lesions in the colon of monkeys. Moreover, agglutinins for *F. necrophorus* are present in the serum of patients with ulcerative colitis, but not in sera from normal persons (Dack, Heinz and Dragstedt 1935, Dack, Dragstedt and Heinz 1936, 1937, Dack *et al.* 1938, Dack *et al.* 1939*a*). These facts, as Dack points out, do not establish *F. necrophorus* as the cause of ulcerative colitis, but indicate that it at least plays a part as a secondary invader of the lesions. It is noteworthy that sulphanilamide has been found to have a beneficial effect in experimental lesions produced by *F. necrophorus* in rabbits (Hemmens and Dack 1939), but not to affect the course of ulcerative colitis in man (Dack *et al.* 1939*b*).

In *animals*, necrobacillosis takes the form of calf diphtheria, necrotic stomatitis of cattle, foot-rot of sheep, gangrenous dermatitis of horses and mules, hepatic necrosis of cattle, pigs, and sheep, labial necrosis of rabbits, and a number of other conditions (see Beveridge 1934).

In **calf diphtheria,** which was studied by Loeffler (1884), a false membrane stretches from the throat down into the trachea; the superficial parts are caseous and friable, the deeper parts firmly adherent. Microscopically the membrane consists of a superficial layer containing large numbers of micrococci: a middle layer, amorphous and unstained; and a deep layer containing granular detritus with some cells and the characteristic long wavy rows of bacilli; these are separated by a narrow unstained zone from a dense infiltration of cells. The lungs contain pneumonic foci, in which much the same microscopical picture is evident. A similar disease to calf diphtheria is said to occur in lambs.

Foot-rot of sheep is usually regarded as due to *Fusiformis necrophorus*, but Beveridge (1935, 1938) in Australia has cast doubt on this. He finds that neither *Fusiformis necrophorus* nor *Treponema penorthum*, which can be seen microscopically in smears from the lesions, are able, alone or together, to set up the disease in healthy sheep. On the other hand, a long, anaerobic, Gram-negative, non-sporing rod having knob-like ends, which was present in smears in only small numbers, was cultivated in a medium containing 25 per cent. of serum, and when applied in pure culture, or better still with *Trep. penorthum*, gave rise to typical foot-rot in healthy adult Merino sheep. Beveridge, who refers to this organism as " organism K," thinks that it belongs to the *Bacteroides* group, but from the description he gives it seems more probable that it is a species of *Fusiformis*. Working with scrapings from lesions, not with pure cultures, Beveridge found that the infecting agent, as judged by its ability to cause foot-rot in healthy sheep when applied to the

scarified area between the digits, might survive for a few days in mud or in sheep fæces, but never for as long as 3 weeks. On the other hand, it could persist for over 3 years in lesions of chronically infected sheep, and for as long as 7 months in superficial skin lesions between the digits. Sheep, however, that had recovered from the disease for a month or more appeared to be no longer infective. Since the causal agent does not survive for more than a short time under saprophytic conditions, it should be possible to free pastures from infection by removal, preferably during the summer, of all infected sheep.

Labial necrosis of rabbits is characterized by a dark bluish-red discoloration of the under lip, accompanied by a tender swelling. The infiltration passes gradually down the under surface of the mouth and front of the neck, and in about 8 days reaches the upper opening of the thorax. Constitutional symptoms develop about the 5th day; a thin watery discharge comes from the nose; the respirations increase in rate; the temperature rises 1–1·5° C. and the animals die in an emaciated condition with marked dyspnœa. Post mortem, section shows that the under lip is converted into a yellowish-white, compact, bacon-like, necrotic mass, which in places extends to the bone; around the necrotic mass is a reddish-black border. The cervical glands are greatly swollen, juicy, and greyish-red; sometimes they show small caseous foci. There is bloody, slightly turbid fluid in the pleural and pericardial cavities with some fibrin deposit on the surrounding serous membranes. A few pneumonic areas may be seen in the lungs, with yellowish-white streaks passing from the pleura into the dark red pulmonary tissue. The spleen and other viscera appear normal. The disease was first described by Schmorl (1891), who observed an epidemic among his laboratory rabbits.

The diagnosis of necrobacillosis is made by morphological and cultural methods. In the lesions the organisms appear mostly as Gram-negative wavy non-branching filaments lying parallel to one another. Attempts to isolate them may be made directly by the inoculation of serum agar shake cultures, or by streaking plates of blood or potato extract agar containing 1/10,000–1/20,000 gentian violet and incubating anaerobically (Slanetz and Rettger 1933). In view of the extreme oxygen sensitivity reported in some species of *Fusiformis*, surface plates should be subcultured as soon as possible after removal from the anaerobic atmosphere. The incorporation in the plate media of reducing substances like thioglycollic acid, which prolongs the life of colonies of oxygen-sensitive clostridia when exposed to air (N. J. Hayward pers. comm.), may prove to be similarly effective with colonies of *Fusiformis*. As an alternative method of diagnosis, a tissue suspension may be inoculated subcutaneously into rabbits, and cultures made from the blood a few hours before death (Scrivner and Lee 1934). The agglutination reaction does not provide a reliable index of infection in animals. Apart from fallacies due to the instability of suspensions of some strains of *Fusiformis* bacilli, titres of up to 1 : 800 are found in the blood of normal adult horses, cattle, sheep and swine (Feldman, Hester and Wherry 1936).

OZÆNA

Ozæna is a disease characterized by atrophy of the nasal mucosa, and the secretion of muco-pus, which dries into greenish-yellow crusts with a peculiarly foul and penetrating odour. It starts generally in childhood or adolescence, and persists throughout life. Though a great many workers have studied the disease, its ætiology still remains doubtful; indeed, it is not clear whether it is an infective disease at all. For a long time there were, among those who supported the in-

fective theory, two rival schools: one, following Loewenberg (1894) and Abel (1896), maintained that the disease was caused by the *B. mucosus ozænæ*—a member of the group of Gram-negative capsulated bacilli (see Chapter 28); the other maintained that the primary cause was Perez's bacillus (Perez 1899, 1901, 1913) (see Chapter 19). A fact in favour of the former group was the isolation of the Loewenberg-Abel bacillus from nearly every case of the disease; in favour of the latter group was the reproduction of a chronic rhinitis by intravenous injection of Perez's bacillus into rabbits.

We do not propose to discuss the evidence for and against the claims of these rival schools; it is sufficient to point out that most of the early workers entirely underestimated the difficulty of establishing the ætiological rôle of any organism isolated from such a situation as the nose. And since diseases such as ozæna and rhinoscleroma have not yet been reproduced with certainty in animals, it is impossible to make out a strong enough case in favour of any one organism to warrant belief in its specific ætiological rôle.

At the present day few workers would care to dogmatize about the causation of ozæna. The general trend of opinion is that the organisms described by Loewenberg, Perez, and others are merely secondary invaders, which are able to multiply abundantly in the altered conditions accompanying an atrophic rhinitis. (For further references see Fricke 1896, Page 1912, Hofer 1913*a*, *b*, Ward 1916, 1917, Shiga 1922, Busson 1923, Blanc and Pangalos 1925, Olinescu and Atineu 1925.)

RHINOSCLEROMA

Rhinoscleroma is a disease of the nose and upper part of the respiratory tract, characterized by proliferative lesions of the infective granuloma type. It is confined to man, and occurs endemically in Eastern Europe and sporadically elsewhere. Most of the reported cases have occurred in Slavs. It would appear to be either non-contagious or only slightly contagious (Perkins 1907). It has not been transmitted to animals.

Bacteriologically rhinoscleroma stands in much the same position as ozæna. In 1882 von Frisch cultivated a member of the group of capsulated bacilli from the lesions; he called it *B. rhinoscleromatis*. By injecting cultures into the submucous tissue of the nasal septum, he produced in rats a small tumour, which contained the organisms (Galli-Valerio 1911). Though Frisch and his followers regarded the disease as caused by this bacillus, there is very little evidence to show that this organism is primarily responsible for it. There is no means by which it can be distinguished with certainty from other members of the capsulated group; and since we know that members of this group may be present in the nose of healthy persons, it is difficult to prove that they play any part in the production of rhinoscleroma. The probability is that they are mere secondary invaders, which grow freely in the nose of patients suffering from the disease.

Tomášek (1925), Quast (1926), and Kouwenaar, Maasland, and Wolff (1934) state that the majority of cases give a positive complement-fixation reaction with a suitable antigen prepared from the rhinoscleroma bacillus, whereas control cases of ozæna and other respiratory diseases fail to do so. The test appears to be of some value in epidemiological survey work.

GRANULOMA VENEREUM

SYNONYM: *Granuloma inguinale*

This disease, which is not to be confused with lymphogranuloma inguinale (see p. 1869), is a venereal disease characterized by a slowly progressive ulceration of the tissues in the genital region. It is widespread in the tropics. Both sexes are attacked. The incubation period varies from a few days to 2 or 3 months. Spontaneous cure is uncommon, but the infection is susceptible to treatment with antimony.

Many years ago Donovan (1905) described the constant presence in smears from the ulcerated lesions of characteristic intracellular bodies, which he regarded as parasites, and which now usually bear his name. They are often contained in giant mononuclear cells. They resemble bacilli of the Friedländer group, are Gram-negative and are surrounded by a well-defined capsule, which can be demonstrated by Wright's stain. Non-capsulated forms, however, are also present. In spite of several claims to have cultivated the causative organism, the precise agent responsible for this disease remained obscure till Anderson, DeMonbreun and Goodpasture (1945) succeeded in cultivating it in the yolk sac of the developing chick embryo. The organism will not grow on ordinary media nor on the chorio-allantoic membrane. It requires some substance present in the yolk of a fertile egg for its nutrition. After a little adaptation it will grow in yolk removed from the embryo and transferred to a test-tube. Yolk from embryos in the early stages of development (4–8 days) is better than that taken later (12–14 days). The growth-supporting substance in yolk withstands heating at 60° C. for 30 minutes. In cultures the organism is moderately pleomorphic, capsulated and uncapsulated forms, long bipolar-stained bacilli, safety-pin forms, and short chains of irregularly stained rods being met with. In sealed test-tubes, cultures may remain viable for 4 or 5 weeks at 25° C. Injection of cultures into a variety of animals, including monkeys, fails to set up progressive disease. Anderson and her colleagues therefore conclude that the causative organism of granuloma venereum is a specific human bacterial parasite having—so far as is known at present—unique growth requirements, and they suggest for it the name *Donovania granulomatis*.

In a further paper, Anderson, Goodpasture and DeMonbreun (1945) describe the preparation from chick embryo yolk cultures of a washed bacterial suspension which gives rise, on intradermal injection into patients suffering from the disease, to a red œdematous reaction reaching its height in 24 hours and disappearing within another 2 days. They were also able to extract with weak alkali a mucoid material, probably of capsular origin, which gave specific precipitin and complement-fixation reactions with the sera of granuloma patients. These results leave little doubt that the true causative organism of this disease has been discovered, and they open the way to a biochemical study of the essential food requirements of this interesting bacterial parasite.

SOFT CHANCRE OR ULCUS MOLLE

Soft chancre is a non-syphilitic lesion of the external genitals and neighbouring regions due to infection with *Haemophilus ducreyi*, a small Gram-negative bacillus first described by Ducrey (1889), and generally known by his name. For

the differential diagnosis of this disease from syphilis by clinical methods, textbooks on venereal diseases must be consulted.

There appears to be little doubt that Ducrey's bacillus is responsible for soft chancre, and for the buboes which are sometimes associated with the primary lesion. Tomasczewski (1903) was successful in reproducing the disease in human subjects with pure cultures. Ulcerative lesions have likewise followed the inoculation of monkeys and rabbits with cultures several generations removed from primary isolation (Reenstierna 1921, Nicolle, 1923). In the diagnosis of the disease, microscopical and cultural examinations should be made in the way already indicated in Chapter 33. An intradermal reaction has been described, and is said to give a high percentage of positive results in infected subjects (Reenstierna 1923, Cole and Levin 1935). The growth from a blood-agar culture is washed off in 0·5 per cent. phenolized saline, and kept in the ice-chest till it is completely sterile. For the test, 0·2 ml. of the suspension is inoculated into the skin. A positive reaction is characterized by the development of a large circular papule with an inflamed border, reaching its maximum in about 48 hours. Care, however, is required in the interpretation of the reaction, since patients who are suffering from lymphogranuloma inguinale may react to the Ducrey antigen, and patients who are suffering from chancroid may react to the Frei test for lymphogranuloma inguinale. A positive reaction, therefore, to one of these tests only is of more diagnostic value than a reaction to both (Greenblatt and Sanderson 1937, Dulaney, 1937).

Vaccine treatment, and treatment with an antiserum prepared by the inoculation of rams, have been stated to yield satisfactory results (Reenstierna 1923, Nicolle and Durand 1924, Hababou-Sala 1925, Watanabe 1939).

GLANDULAR FEVER

SYNONYM: *Infectious Mononucleosis*

Glandular fever is a disease of human beings that was first described by Pfeiffer in 1889. Türk (1907) recognized the peculiar lymphoid blood picture, and Sprunt and Evans (1920) suggested the alternative name of infectious mononucleosis. It is an acute infectious disease characterized by fever, enlargement of the lymphatic nodes and spleen, angina, and changes in the lymphocytic cells of the blood. Acute abdominal symptoms, follicular conjunctivitis, jaundice, and a rash, usually of the rubella type, are sometimes observed. The duration of the disease is variable. Relapses are not infrequent; but fatal cases are rare. No fewer than five different clinical types of the disease have been described by Baldridge, Rohner and Hansmann (1926). Tidy (1934) divides it into three clinical types according to whether the glandular enlargement, the sore throat, or the fever predominates. Abortive and ambulant cases are very common. Children and young adults are mainly attacked. The incubation period is about 7 to 12 days, but may be as long as 21 days. The disease is contagious and may occur in epidemic form (see Tidy and Morley 1921, Tidy and Daniel 1923); but the infectivity of sporadic cases is said to be low (Glanzmann 1930).

The *cause* of the disease is still open to doubt. Schmidt and Nyfeldt (1938) described five cases of infectious mononucleosis accompanied by meningo-encephalitis, and found *Erysipelothrix monocytogenes* (*Listerella*) in the blood or cerebrospinal fluid of four of them. Pons and Julianelle (1939) also isolated the same organism

from the blood of a patient, and Webb (1943) records a similar finding. With few exceptions, however, subsequent workers have failed to confirm these observations. Wising (1942), in particular, made a very careful study of 27 typical cases of the disease, but neither from the blood in the acute febrile stage nor from excised lymph nodes was he able to cultivate this organism. Convalescents from glandular fever contained no agglutinins in their blood to *Ery. monocytogenes*. Inoculation of killed *Ery. monocytogenes* into human volunteers did not give rise to a Paul-Bunnell reaction (see below); and inoculation of the living organisms into monkeys led to a rapidly fatal septic infection accompanied by suppurative meningitis and encephalitis, which was unlike typical glandular fever in man.

On the other hand, evidence is accumulating to suggest that the disease is caused by a filtrable virus having a predilection for lymphatic tissue. Van den Berghe and Liessens (1939*a*, *b*) and van den Berghe, Liessens and Kovacs (1939) were able to produce a circulating monocytosis in a monkey by intramuscular inoculation of 4 ml. of oxalated blood from a child suffering from glandular fever, and to reproduce a similar result in a second monkey inoculated with blood from the first monkey after it had been hæmolysed and filtered through a Seitz EK disc. The virus was grown in tissue culture, and still proved capable after ten generations of causing a monocytosis in monkeys. Mice inoculated intracerebrally proved insusceptible. Nettleship (1942) described the production of pearl-grey nodules with heavy monocytic cell infiltration in the chorio-allantoic membrane of chick embryos inoculated with Berkefeld filtrates of nasal washings or with whole blood of glandular fever patients. Wising (1942) failed to reproduce the disease in rabbits inoculated intravenously with heparinized blood of febrile patients, but had some success with monkeys. Of seven *Macacus* or *Cercopithecus* monkeys inoculated intracerebrally, intraperitoneally, or subcutaneously with fresh lymph node suspensions from patients in the acute stage of the disease, three developed mild clinical symptoms with general enlargement of the lymph nodes, coming on after 8, 17 and 19 days respectively. There was also a slight monocytosis. These changes were reproduced in fresh monkeys by inoculation of excised lymph nodes; 2, 3 and 5 passages were made successfully with the three different strains. Histological examination of the nodes revealed the typical irregular pleomorphic and large-celled hyperplasia seen in man. Of six human volunteers inoculated with heparinized blood or plasma from patients in the acute stage, five remained well. The sixth, who had received 250 ml. of blood, developed typical glandular fever 18 days later.

These results are by no means conclusive. It may be that, as Schultz (1930) and Höring (1933) think, glandular fever is a name covering two or three different diseases. One of these, probably the commonest type, may be caused by a virus; another, probably much less common, may follow infection with *Erysipelothrix monocytogenes*. Further investigations, however, are required to establish this thesis.

Laboratory diagnosis is made by cytological examination of the blood and by the Paul-Bunnell test. Though there is usually a relative lymphocytosis, it is not the absolute nor the relative numbers of the lymphocytes that is characteristic so much as the special morphology of the cells. Blood changes may be present even on the first day of fever, but they do not usually reach their maximum till the end of the second week. They persist for weeks or months. The ability of the blood of glandular fever patients to agglutinate sheep red corpuscles was

described by Paul and Bunnell (1932). Normal agglutinins, rarely exceeding a titre of 1/56, may be found in healthy persons (see Smeall 1942), and the heterophile antibody content may rise after injection of horse serum. Observations by Stuart and his colleagues (1934), Bailey and Raffel (1935), Davidsohn (1937), Kristensen (1938), Barrett (1941), and Kilham and Steigman (1942) have shown that the normal heterophile antibody can be absorbed completely from the serum by guinea-pig kidney, that that met with in serum disease can be absorbed by guinea-pig kidney and ox red blood corpuscles, and that the heterophile antibody of glandular fever can be absorbed by ox corpuscles but not by guinea-pig kidney. The Paul-Bunnell test, particularly when confirmed by absorption, is of considerable value in diagnosis. A titre of 1/80 or over is highly suggestive of infection, though some workers, such as Halcrow, Owen and Rodger (1943), would put it as low as 1/32. The agglutinin content often continues to rise for 2 or 3 weeks. Not all cases, however, give a positive reaction. Shafar and Weir (1943), for example, have described an outbreak of glandular fever in Scotland affecting about 200 persons, in which the Paul-Bunnell reaction was almost uniformly negative. McFarlan and Macfarlane (1943) likewise have described an outbreak in a girls' school affecting 78 of the pupils, in which typical blood changes were present but in which not a single serum reacted positively in the Paul-Bunnell test. The explanation of this is doubtful. It may be that there is more than one type of the disease, or that in some cases the amount of virus in the circulating blood is too small to react in the presence of sheep red corpuscles; or that only the first stage of the antigen-antibody reaction occurs (see Levine and Gilmore 1945). The mechanism of the reaction is likewise still doubtful, but it may be similar to that of the Hirst test in influenza (see p. 1658); if so, then the antibody titre may be an index of the amount of circulating virus.

Treatment of the disease by the sulphonamides has not so far proved encouraging. (For references to papers and reviews on glandular fever see Tidy and Morley 1921, Baldridge *et al.* 1926, Schwarz 1929, Glanzmann 1930, Tidy 1934, Anton 1934, McKinlay 1935, Wising 1942, Halcrow *et al.* 1943.)

INFECTIVE HEPATITIS

SYNONYMS.—Epidemic jaundice; common infective jaundice.

Infective hepatitis is a disease that tends to occur in epidemics. Clinically it is characterized by a sudden onset with vomiting, fever, malaise, and sometimes abdominal pain. Jaundice develops in a few days, being apparent first in the conjunctiva, and then spreading to the rest of the body; frequently it gives rise to itching. The urine is dark and the stools are pale. Bradycardia is common and the liver is often enlarged and tender. The disease lasts from a few days to three weeks or more, but complete recovery may take some months. Relapses may occur, but are not common. The morbidity varies greatly. The disease may be confined to sporadic cases, or it may affect up to 50 per cent. of the exposed population. Abortive and ambulant cases are not uncommon, and there is evidence that healthy carriers may occur. The sexes appear to be equally susceptible. The disease is most frequent in children of school age, but young adults are also affected. The incubation period is generally 3 to 5 weeks, though Sheehan (1944) maintains that it is probably 3 months. The period of infectivity appears to be

confined to the first week or two at most of the disease. Multiple cases in a household, sometimes occurring at approximately 4-week intervals, are often observed. As a rule, the case mortality is not more than about 0·1 per cent., but in some localized outbreaks quite a number of the patients may die. The histological appearance of the liver at autopsy, or as revealed by biopsy (Roholm and Iversen 1939), is one of diffuse lobular necrosis with mononuclear infiltration and a varying degree of connective tissue proliferation, but without involvement of the bile ducts.

The disease is supposed to be conveyed by droplet infection, but one water-borne outbreak has been described by Fraser (1931) in Canada, and one milk-borne outbreak in Scotland, affecting about 150 school children (Report 1943*b*). Andersen (1937) and Andersen and Tulinius (1938) in Denmark thought that infection occurred usually by the alimentary tract, and that the disease was ætiologically related to epidemic jaundice in pigs. The observations, however, on which these workers based their conclusions have not been confirmed. Sheehan (1944) quotes evidence suggesting that the disease is borne by insects—possibly bed bugs. On the whole the evidence suggests that infection is usually conveyed by droplet nuclei from the nasopharynx or by infected dust, which are inhaled or come into close contact with the retropharyngeal tissues during ingestion. The ætiology of the disease is still obscure. So far, no micro-organisms have been identified as a likely cause. Mention, however, should be made of the water-borne outbreak of jaundice described by Fraser (1931), in which jaundice followed 3–5 weeks after an outbreak of acute gastro-enteritis caused apparently by *Salm. paratyphi B* or some closely related organism of the *Salmonella* group. German workers (Siede and Meding 1941, Mancke and Siede 1942, and Siede and Luz 1943) claim to have cultivated a virus from the duodenal juice of patients on the chorio-allantoic membrane of the developing chick embryo, but attempts by Hoyle (1943) and others in this country to repeat their observations have been unsuccessful.

Though a number of workers have endeavoured unsuccessfully to transmit the disease to a wide variety of animals (Findlay, MacCallum and Murgatroyd 1939, van Rooyen and Gordon 1942), human volunteer experiments have been more promising. Admittedly, the results have been irregular, but there is now little doubt that an infective agent has been demonstrated in the serum, the nasopharynx, the duodenal juice and the fæces of patients in the pre-icteric or early icteric stage of the disease (Voegt 1942, Cameron 1943, MacCallum and Bradley 1944).

In discussing the ætiology of infective jaundice, it is important to draw attention to certain other types of jaundice. *Catarrhal jaundice*, which is believed to result from obstruction of the common bile duct, caused either by cholangitis or by pressure, is a sporadic, non-infective disease, and has probably no relation to infective hepatitis. Serum jaundice and arsenic jaundice, on the other hand, may be closely related to it.

Serum jaundice has been observed in man and in horses inoculated with homologous serum, usually for the prophylaxis of some disease. In 1937 a small outbreak occurred among the recipients of a single batch of measles convalescent serum (Report 1938*b*). Propert (1938) described a similar outbreak following the prophylactic use of serum for measles in an institution for mental defectives. Findlay and MacCallum (1937, 1938) in this country and Soper and Smith (1938) in the United States reported its occurrence after the use of yellow fever vaccine. In Brazil there was an attack rate of 27 per cent. among 304 persons immunized

with a particular batch of yellow fever vaccine (Fox *et al.* 1942); and during the first half of 1942 there was a very large outbreak in which 28,585 cases occurred in American troops who had received yellow fever vaccine (Report 1942*a*, *b*). In all these instances the vaccine had included human serum in its preparation. Jaundice has also followed inoculation with mumps convalescent serum (see Beeson *et al.* 1944). Observations have shown that with some batches of serum as small a quantity as 0·1 ml. may prove infective (see Bradley, Loutit and Maunsell 1944). Horses injected with immune horse serum in an attempt to prevent such diseases as grass sickness, equine encephalomyelitis, staggers, and anthrax have developed jaundice (for references see Report 1943*a*). The incubation period in serum jaundice is variable, but is usually about 2 to 3 months. The morbidity is often high. The case mortality in some outbreaks has been negligible; in others it has been considerable. There is at present little evidence to suggest that serum jaundice is contagious. Oliphant, Gilliam and Larson (1943) have shown that the serum of patients suffering from jaundice after the inoculation of yellow fever vaccine can itself give rise to jaundice when injected subcutaneously into human volunteers; and that the blood of the donor may be infective before the appearance of the jaundice, but not 2½ months after its disappearance. Moreover, icterogenic serum may give rise to jaundice when given by the mouth in gelatin capsules; the incubation period under these conditions may be as short as 30 days (see Havens *et al.* 1945). Findlay, MacCallum and Murgatroyd (1939) regard the agent of serum jaundice as a virus on the grounds that it can be propagated in tissue culture, it fails to grow on ordinary bacteriological media, it is invisible under the microscope, it passes freely through a Seitz EK filter, it withstands freezing and drying for many months at 4° C., and it gives rise to a disease characterized by an incubation period varying from 35 days to 7½ months. In this connection it may be pointed out that a long incubation period is not uncommon in some virus diseases, such as rabies, infective warts, and the disease of sheep known as scrapie, in which it may extend up to 2 years. Growth in tissue culture, however, is very doubtful, and has not been satisfactorily established.

Arsenic jaundice may occur during the treatment of syphilitic patients with salvarsan, neosalvarsan, arsphenamine, or other similar drug. Ruge (1931), whose monograph should be consulted, studied large numbers of cases in the German navy during the years 1919–29. The incubation period varied between 2 and 3 months according to the nature of the arsenic preparation used. Four-fifths of the cases occurred in men under 26 years of age, and 99·5 per cent. of the cases had a negative Wassermann reaction at the time the jaundice appeared. The case incidence was higher in men on board than on shore, and in small ships than in large, suggesting overcrowding as a predisposing factor. The frequency of arsenic jaundice rose and fell almost simultaneously with that of ordinary infective hepatitis. Fresh light was thrown on the disease when it was found that infection could be transmitted from patient to patient by the use of imperfectly sterilized syringes used for intravenous inoculation. Even a trace of blood left in the syringe from an infected patient during the incubation period may suffice for this purpose (Sheehan 1944, Salaman *et al.* 1944). How far arsenic jaundice is due to the toxic action of the drug on the liver, and how far to a virus transmitted by inoculation from one patient to another—the arsenic therapy being mainly incidental—it is at present impossible to say.

Though serum jaundice and arsenic jaundice differ from infective hepatitis

in their longer incubation period and in their apparent lack of infectivity for contacts, they resemble it in several ways. The general symptomatology, the changes in the white cells of the blood, the primary necrosis of the liver (see Dible *et al.* 1943), and the negative bacteriological findings suggest that these three diseases may all be manifestations of the same primary infective agent. This view is strongly maintained by Sheehan (1944), who believes that the incubation period of epidemic hepatitis is 3 months, and not 4 weeks as is usually held.

Even though there is a difference in the usual length of the incubation period, as appears probable from numerous reliable observations, there is no justification for concluding that infective hepatitis is caused by a different virus from that responsible for serum and arsenic jaundice. In these two diseases the virus is inoculated along with serum, and it may be that antibodies present in the serum delay its multiplication. In infective hepatitis, on the other hand, the virus gains direct access to the tissues, and its invasion of the body may therefore occur more rapidly. Much active research is now in progress on this interesting group of diseases, and crucial experiments are being undertaken to determine whether a patient who has recovered from one disease is immune to either of the other two. So far as it is possible at present to draw any conclusions, we feel that the balance of evidence favours the view that these three diseases are caused either by the same agent or by closely allied agents—probably belonging to the group of filtrable viruses.

Apart from exclusion of a known pathogenic agent and of Weil's disease, bacteriology can be of little help in the diagnosis of infective jaundice. Though there may be a transient leucocytosis in the early stage of the disease, leucopenia is the rule. There is a rise in the relative proportion of monocytes, but less than that in glandular fever. The blood picture alone is often of value in the differential diagnosis from Weil's disease, in which there is a polymorphonuclear leucocytosis. A complement-fixation test with the patient's serum has given promising results, but is still too much in the experimental stage to be recommended for routine use.

Beyond the usual general measures taken to prevent the spread of respiratory infections, there is little of known prophylactic value to recommend. The duration of infectivity of the patient appears to be short, and probably two weeks' isolation is ample (see Pickles 1939, Edwards 1943). (For further information see Blumer 1923, Wollenberg 1926, Klemperer, Killian and Heyd 1926, Hurst and Simpson 1934, Wickström 1936, Report 1938*a*, Pickles 1939, Cullinan 1939, McFarlan 1941, 1945, Newman 1942, Findlay, Martin and Mitchell 1944.)

BARTONELLA INFECTIONS. OROYA FEVER. VERRUGA PERUANA. INFECTIOUS ANÆMIA OF RATS, DOGS, AND MICE

These diseases, which have attracted attention during recent years, are caused by minute micro-organisms intimately associated with the red blood corpuscles, and belonging either to the *Bartonella* or *Eperythrozoon* groups (see Chapter 37).

Oroya fever and **Verruga peruana** appear to be two stages of the same disease. They are limited to the tropical zone of western South America, namely Peru, Colombia and Ecuador. Owing to the widespread epidemics that may occur and the high case mortality of the disease—somewhere about 40 per cent.—Oroya fever is to be regarded as one of the most serious infections of South America.

In the Colombian outbreak of 1938, 4,000 deaths occurred among a total population of 100,000 (see Weinman 1944).

Oroya fever, sometimes known as Carrión's disease, is characterized by fever and a severe progressive anæmia, the red cell count sometimes falling within a few days to 1 million per c.mm. Examination of the blood shows the presence in the red corpuscles of small rod, dumb-bell, and coccoid bodies, varying considerably in number and staining red or reddish-purple with Giemsa. The organisms can be cultivated on semi-solid nutrient media. Inoculated intravenously into young *rhesus* monkeys, pure cultures of *Bartonella bacilliformis*, but not infected blood, give rise to an irregularly remittent type of fever, sometimes accompanied by severe anæmia. The incubation period of the disease is usually about 20 days. In fatal cases death occurs as a rule in 3 to 4 weeks. In patients that recover, convalescence is established after about 5 or 6 weeks, but is often succeeded in a month's time by an eruption of verruga. Second attacks of Oroya fever appear to be uncommon.

Verruga peruana is a disease characterized by the appearance on any part of the body surface of vivid red wart-like eruptions. The disease follows a short time after an attack of Oroya fever, and lasts as a rule for 4 to 6 months. Second attacks may occur, and sometimes a latent infection persists after the subsidence of the skin lesions. The case mortality is very low. There is generally a moderate degree of anæmia, but the parasites in the blood are too few to be seen microscopically, though they can often be revealed by culture. *Bartonella* can be readily cultivated from the local lesions, and can be seen microscopically in stained sections of the excised tissues. Pure cultures, or juice from the nodules, inoculated intradermally above the eyebrow of monkeys give rise after an incubation period of 9–20 days to a local verruga papule. Experimental infection is followed by immunity. In the monkey the spleen appears to play no part in the defence mechanism of the host, such as it does in the rat infected with *Bartonella muris*.

Both Oroya fever and verruga appear to be carried by sandflies, such as *Phlebotomus noguchi* and *Phlebotomus verrucarum* (Shannon 1929, Hertig 1937). In endemic areas latent infections in man appear to be not uncommon (Weinman and Pinkerton 1937). The blood of patients suffering from the disease may contain agglutinins to *Bartonella bacilliformis* in a titre of 1/10 to 1/80 (Howe 1942). Organic arsenic compounds have no curative effect. (For further description see Noguchi 1926, Kikuth 1931, 1934, Pittaluga 1938, and a comprehensive review by Weinman 1944.)

Infectious Anæmia of rats, caused by *Bartonella muris*, is a disease that is precipitated by splenectomy. With the exception of certain breeds, such as the Wistar strain, a large proportion of adult rats, both wild and tame, appear to suffer from a latent infection with this organism. In such animals removal of the spleen is followed, usually in 4 or 5 days, by general illness, emaciation, and a severe progressive anæmia. Frequently hæmoglobinuria develops and the animal dies, generally within 14 days. Recovery may, however, occur, but it is liable to be followed by relapses at irregular intervals. Examination of the blood during the acute stage of the disease reveals a high proportion of red cells infected with *Bartonella* (Fig. 213, p. 904). The organisms have been cultivated successfully on leptospiral and other media. In animals that recover they disappear from the blood in 1–5 weeks. Splenectomy in young rats that have not yet become infected with *Bartonella* is without effect, but if a pure culture or infected blood is injected into a splenectomized animal then typical anæmia develops. Inoculation of a

non-infected non-splenectomized animal produces only a mild anæmia. The disease can also be reproduced in young rabbits, guinea-pigs, and white mice by inoculation.

The part played by the spleen is decisive, though in what way it acts is still a matter of conjecture. A quarter of the spleen left *in situ* is sufficient to protect the animal against the disease (Perla and Marmorston-Gottesman 1930). Perla and Marmorston-Gottesman (1932) have prepared an aqueous lipoid extract of the spleen that neutralizes the effect of splenectomy. The same workers find that if rats are fed prior to splenectomy on a diet containing an adequate amount of iron and copper, a large proportion of them fail to develop anæmia. Sandberg and Perla (1934) have shown that splenectomy in non-infected albino rats is followed by an increased retention of iron but an increased elimination of copper. The suggestion is therefore that the spleen plays an important part in the utilization of copper, and that in animals on a normal diet the amount of this substance is insufficient to prevent the development of a severe anæmia if the spleen is removed. Certain trypanosome infections, poisons such as toluylenediamine, pyridine, and phenylhydrazine (Lauda and Marcus 1928), irradiation, and possibly some bacterial infections, may activate a latent *Bartonella* infection even in the presence of an intact spleen.

Natural infection is probably carried by rat lice. Neosalvarsan administered before splenectomy prevents the development of anæmia. Administered after the disease has developed, it has a marked therapeutic effect. Certain arsenic-antimony compounds are said to be even more effective. According to Emery (1940), sulphanilamide has no therapeutic action. (References: Mayer 1921, Ford and Eliot 1928, Eliot and Ford 1929, Perla and Marmorston-Gottesman 1930, 1931, 1932, Marmorston-Gottesman and Perla 1930, 1931, 1932*a*, *b*, Lwoff and Vaucel 1931, Roth 1932, Kikuth 1934, McCluskie and Niven 1934.)

Infectious Anæmia of dogs is caused by *Bartonella canis*. The organisms were first observed by Kikuth (1929) in the blood of a splenectomized dog. The course of the disease resembles that in rats. The parasites multiply up to a certain point, then suddenly disappear from the peripheral blood, only to reappear after a few days and again work up to a maximum. Remissions of this type may occur for months. The length of the relapses and of the remissions varies from dog to dog (Regendanz and Reichenow 1932). The anæmia is seldom severe; death is infrequent; and spontaneous recovery leaves behind it a substantial degree of immunity. Infection appears to be transmitted from dog to dog by fleas. Neosalvarsan exercises a specific effect on the course of the disease. At the height of the disease *Bartonella canis* is present in very large numbers. It is said by Regendanz and Reichenow to be situated extracellularly. This may account for the fact that the parasites may suddenly disappear from the blood without any serious diminution occurring in the number of red blood corpuscles. The organisms are very pleomorphic, and may become agglutinated into characteristic masses or chains at the periphery of the cells. Cats, as well as dogs, are susceptible to experimental inoculation after splenectomy (see also Pérard 1929, Lwoff and Provost 1929, Kikuth 1934).

HYPEREMESIS HIEMIS: THE WINTER VOMITING DISEASE: EPIDEMIC NAUSEA AND VOMITING

The winter vomiting disease has been met with in many countries. Outbreaks have been reported by Zahorsky (1929, 1940) in the United States, by Rischel

(1935) and Henningsen (1936) in Denmark, and by Miller and Raven (1936), Gray (1939), and Bradley (1943) in Great Britain. It is possible that the outbreak of gastro-enteritis described by Smith and Davies (1941) was an example of the same disease. Whether it is related to the vomiting sickness of Jamaica, which was ascribed by Scott (1916) to poisoning by achees, is very doubtful, but in view of the benign nature of the winter vomiting disease and the high case mortality of the Jamaican sickness, this seems improbable. Zahorsky first experienced an epidemic at St. Louis in 1904. He observed it during many subsequent winters. The last outbreak he describes was at St. Louis in 1940, when fully 3,000 children were attacked during the first three weeks of February. A similar outbreak at Charleston, S.C., reported by Waring (1942), affected several thousand persons during February and March 1941. All ages are attacked, but infants and young children seem to be specially susceptible. The incubation period is thought to be 2 to 7 days. The onset of the disease is often abrupt, but it may be preceded by abdominal pain or nausea for a day or two. The vomiting is violent, forcible, and often projectile. It lasts for a day or two, and may lead to a state of collapse. Soon after its commencement diarrhœa, accompanied sometimes by colicky pains, sets in. It is seldom profuse, though it may be so. The stools are thin, pale in colour, and very offensive. A temperature of 99°–100° F. is not uncommon. Children sometimes appear profoundly ill. They remain, without food, in a listless or semi-comatose state for 2 or 3 days. Death, however, is rare. A mild inflammation of the upper respiratory tract is often present. The leucocyte count tends to be raised. The clinical diagnosis is made on the presence of an epidemic, persistent vomiting, offensive light-coloured stools, the relatively afebrile course, and the absence of an acute respiratory infection or otitis media. The cause of the disease is unknown. Our own observations, like those of others, have failed to reveal the presence of any known pathogenic organism. The practical absence of cells from the stools suggests that the disease is not primarily an enteritis, nor do epidemiological studies point to a food-borne infection. It seems more probable that infection occurs through the respiratory tract. Zahorsky (1940) distinguishes it from influenzal gastritis, since it may supervene explosively on an outbreak of this disease.

INFECTIOUS CATARRH OF MICE

Nelson (1937) has described a disease of mice characterized by rhinitis, otitis media, and a terminal lobar pneumonia. Infection spreads readily by contact, progresses slowly, but ultimately proves fatal. No nasal discharge is visible externally, but a copious semi-fluid white exudate can be withdrawn from the nostrils by a capillary pipette. The disease can be readily transmitted to normal mice by instillation of exudate from the nose, ear or lung. Microscopical examination of nasal or ear discharge reveals the presence of small Gram-negative coccobacilliform bodies, 0·3–0·4 μ in diameter, arranged singly, in pairs, or small groups, and predominantly extracellular. The organisms cannot be cultivated on ordinary media, but can be grown in tissue culture. Nasal instillation of pure cultures reproduces the disease in mice. The coccobacilliform bodies are similar to those found by Nelson (1936) in fowl coryza. Whether infectious catarrh of mice is allied to the so-called mouse-influenza described by Kairies and Schwartzer (1936) in Germany is not known.

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CHAPTER 80

RELAPSING FEVER, AVIAN SPIROCHÆTOSIS, AND VINCENT'S ANGINA

RELAPSING FEVER

SYNONYMS : Famine fever ; tick fever.

RELAPSING fever is the name given to a disease the chief characteristic of which is the occurrence of one or more relapses following the subsidence of the primary febrile paroxysm. It would perhaps be more correct to define it as a general term for a group of closely allied diseases ; for relapsing fever differs in its symptomatology in different countries. The disease is widespread in Eastern Europe, in Asia, in Egypt, and along the North African coast, in Mexico, and in Central and South America. It used to occur in this country and in Ireland, particularly during times of famine ; the last epidemic was in 1868–71. In the United States the earliest record is of an outbreak in Philadelphia in 1844 among immigrants from Liverpool. Further outbreaks among immigrants were recorded in New York in 1847 and 1871, and in Philadelphia in 1869 (Moursund 1942). There was an outbreak in 1874 among Chinese labourers in California, though according to Meyer (1936) this was more probably typhus fever. The first indigenous cases were recognized in 1915 in Colorado ; since then there have been numerous reports of the disease in the south-western States including California. (For epidemiology in United States, see Report 1942.) The tick fever of Central Africa was shown simultaneously by Ross and Milne (1904) and Dutton and Todd (1905) to be a form of relapsing fever. The incubation period is from 2 to 10 days as a rule, sometimes as long as 14 days. The mortality is generally low—less than 6 per cent.—but in some epidemics it has been as high as 30 per cent. The causative organism of the disease was first observed by Obermeier in the Berlin epidemic of 1867–68 ; his observations were published in 1873 (Obermeier 1873). He noticed the presence of thread-like bodies in the blood of patients during the febrile stage of the disease, the disappearance of these bodies during the afebrile stage, and their reappearance during the relapse. By reason of their motility and their wave-like spiral form, he considered them to belong to the group of Spirochætes. In the blood they occurred as straight forms, S-forms, and even as circles ; towards the end of the paroxysm he noticed their tendency to aggregation in rosettes and their granular disintegration. The organisms occurred only in patients with relapsing fever ; they were never found in the blood of normal persons, or of patients suffering from other fevers. These observations are of considerable historical importance, because they were among the first to establish the microbial theory of infection.

The spirochæte described by Obermeier is known as *Treponema recurrentis*, and is the usual type found in European relapsing fever. Besides this, a number

of other spirochætes have been described associated with relapsing fever in other countries; and, as these exhibit certain differences, particularly in their antigenic structure, their virulence to various laboratory animals and, perhaps most important of all, their specificity for their intermediate host—the louse or the tick—they have been regarded by some workers as separate species and named accordingly. Thus we have *Trep. duttoni* of Central Africa, *Trep. novyi* of North America, *Trep. kochi* of East Africa, *Trep. carteri* of India, *Trep. persicum* of Iran, *Trep. usbekistanicum* of Russian Turkestan, *Trep. sogdianum* of Palestine, *Trep. hispanicum* of Spain, *Trep. venezuelense* of South America, and a few more. Other workers, however, regard all these organisms as varieties of the same species.

As seen by dark-ground illumination in the blood of patients during a febrile paroxysm, they are actively motile spiral organisms, with a series of five to ten fairly regular but loose primary waves; during rest their axis is generally straight, but when in motion they momentarily assume various curved and bizarre forms. Their length is variable, and differs with different species; generally it is 10–20 μ. The spirals are 2–3 μ long, and about 1 μ in amplitude. Division occurs by transverse fission; during this process a constriction appears at the middle, and the two organisms draw apart, leaving a thin thread-like connection between them. After separation has occurred, this remnant of the periplast may often be seen attached to one end; by many workers it has been described as a flagellum. Under suitable conditions the organisms are extremely motile, darting rapidly across the field; but in ordinary wet blood films their motion is slower; they move backwards and forwards over a distance of not more than two or three times their own length (Novy and Knapp 1906). Rotation occurs around the long axis. The numbers present in a blood film vary from case to case; at the height of the first pyrexial attack they are often numerous—several organisms to a field—but they may be relatively few and difficult to find. During the decline of the fever their numbers diminish, the organisms become less motile, and not infrequently they assume irregular shapes or accumulate in rosettes (see Fig. 215, p. 913); these changes are regarded as indicative of lysis or agglutination due to the action of antibodies developing in the host. After the subsidence of the fever they can no longer be found microscopically in the blood; there is evidence, however, to suggest that a few organisms may persist in the blood, since inoculation of blood into animals during the apyrexial interval may give rise to infection. At the onset of the relapse they again become demonstrable microscopically in the blood, though not always in such large numbers as in the first attack. During the interval between the pyrexial attacks the organisms remain latent in the tissues. Experiments conducted on mice suggest that the brain is one of the organs in which infection frequently persists (Heronimus 1928).

The spirochætes are best demonstrated by dark-ground illumination; but they may be stained by methylene blue, or preferably by Leishman or Giemsa, with both of which they take on a bluish colour. If the blood films are made in the usual way and allowed to dry in air, the organisms undergo gross distortion and present irregular and coiled forms; if their natural form is to be preserved, they should be wet-fixed.

Transmission of the Disease.

Relapsing fever is transmitted by blood-sucking insects. Dutton and Todd (1905) showed that the tick fever of the Congo Free State was spread by the tick

Ornithodoros moubata; infected ticks were able to transmit the disease to monkeys, and in one experiment young ticks, newly hatched in the laboratory from eggs laid by infected parents, successfully conveyed the disease to monkeys, showing that the infection could be transmitted hereditarily. In Uganda, Ross and Milne (1904) brought evidence to show that the disease was spread by the bite of the tick *Ornithodoros savignyi*. In Panama and South America it is spread by the ticks *O. talaje* and *O. venezuelensis*, in Texas by *O. turicata*, in California by *O. hermsi* and *O. parkeri* (see Davis 1942), in Spain by *O. marocanus* and *O. erraticus*, and in Iran and Palestine by *O. papillipes* (see Delpy and Rafyi 1939). The method by which the tick transmits infection is still subject to doubt. It is generally assumed that the infected fluid exuded by the coxal glands contaminates the wound made by the bite of the tick. In some ticks, however, such as *O. hermsi* and *O. turicata*, the coxal fluid is not infective (Herms and Wheeler 1936, Francis 1938), and infection probably results from the bite itself, particularly from bites of the larvæ. After a good meal of blood, ticks may survive starvation and remain infective for as long as five years (Francis 1938). The mammalian reservoir of infection is constituted by a variety of animals, especially rodents, but including pigs, porcupines, opossums, and armadillos (see Sautet 1937).

Though the disease in Central and South Africa, in North and South America, in Palestine and Iran, and some other parts of the world is spread by ticks, in most of Europe and Asia the chief vector is the louse (Wenyon 1926). The organisms may be demonstrated for 24 hours in the stomach of the louse following an infective feed; after this time they can no longer be found; but in about 6 or 8 days they reappear in the fluids of the body cavity, and spread to all parts of the body including the legs and antennæ. Infection is conveyed to human beings not by the bite of the louse, which appears to be harmless, but by contamination of the wounds, made by biting or by scratching, with the body fluids of the louse. In ticks, Wheeler (1942), working with *O. hermsi*, found that the spirochætes invaded the cœlomic cavity within three days of being ingested, and by the tenth day were demonstrable in the muscles. In contrast to ticks, hereditary transmission of infection has never yet been satisfactorily demonstrated in lice. Occasionally human beings are infected by contamination of a wound, or even of the nasal or conjunctival mucosa, with the blood of an infected animal. Laboratory infections of this kind are not uncommon.

Immunity.

The disappearance of the spirochætes from the blood stream at the end of the first pyrexial attack has been regarded by many workers as due to the development of antibodies. This view has to some extent been substantiated by the work of Novy and Knapp (1906).

Working with the rat, they found that the organisms would live in defibrinated blood kept at room temperature for 30 or 40 days, provided the blood was removed from the animal during the early stage of infection; if it was taken during the later stages, they died in 24 hours. When the organisms were examined *in vivo* during the decline phase of the infection they were observed to become sluggish, to show end-to-end agglutination, and even to form small tangles. This was even more marked when blood from an immune animal was inoculated into an infected rat; half an hour later the spirochætes were accumulated in tangled masses of 10 to 20 members, and showed end-to-end agglutination; 1 hour later they were agglutinated into perfect radiating rosettes, and 2 hours

later they were very scarce and were mostly immobile. Blood serum from a rat, which had been hyperimmunized by a course of 26 injections of infective blood, had marked immobilizing and agglutinative properties, even when diluted to 1/100. In rats that had recovered naturally from infection Pfeiffer's phenomenon could be produced *in vivo*. Intraperitoneal injection of infective blood into such animals was followed by agglutination and granular degeneration of the spirochætes; in 10 minutes no free spirochætes could be found. The altered organisms were rapidly ingested by phagocytes. In hyperimmunized rats the spirochætes completely disappeared from the peritoneal cavity in 2 minutes. In passively immunized rats the spirochætes were agglutinated into rosettes, but later these broke up, and free organisms once more became numerous; the animals, however, did not contract infection. The course of antibody production in human beings seems to resemble that in experimental animals (see Cunningham and Fraser 1935).

From these and other experiments, it would appear that during the course of the natural disease immune bodies—chiefly agglutinins, spirochætocidins and lysins—are developed, which are sufficiently powerful to overcome the blood infection and lead to the disappearance of the spirochætes from the circulation. These organisms remain latent in the brain and other tissues, and when the circulating antibodies have decreased, they once more enter the blood and give rise to a relapse. This stimulates the production of fresh antibodies, which again lead to the disappearance of spirochætes from the blood. After one or more relapses the active immunity developed by the host is sufficient to prevent further invasion of the blood by the spirochætes, and an apparent cure results. Whether a true cure results, in the sense that the body is completely rid of spirochætes, is doubtful. Animal experiments suggest rather that, even though the organisms give no token of their presence, they may yet remain alive in the tissues for weeks or months. Immunity in relapsing fever appears to be an infection-immunity; this corresponds to a state of the host in which, together with a humoral immunity, there is a latent infection of the tissues, which is capable under certain conditions of breaking down the existing immunity (Heronimus 1928). It is a state in which a working equilibrium is established between host and parasite, and like other equilibria is liable to disturbance (see Chapter 51).

There is a considerable amount of evidence to show that after the first attack the spirochætes in the tissues undergo an antigenic change which renders them insusceptible to the antibodies produced by the host against the original strain. This enables them to invade the blood a second time and give rise to a relapse. The production of antibodies to the so-called "relapse" or "serum-fast" strain is followed by the disappearance of the organisms from the blood once more. A further antigenic change may occur in the tissues enabling the organisms to invade the blood for a third time. In some instances it appears that the relapse strain reverts to the parent type, and if the circulating antibodies generated during the first attack have diminished sufficiently, another invasion of the blood may occur (Aristowsky and Wainstein 1929*a*).

It will be understood that the antigenic specificity of the relapsing fever spirochætes is highly developed. This accounts not only for the fact that patients who have been infected with one type of relapsing fever, for example the European, can be infected with another type, such as the Indian or African, but that, as just noted, a patient who has recovered from invasion with a given strain is liable to suffer from a relapse due to an antigenic variant of the same strain.

Reproduction of the Disease in Animals.

Infection can be transmitted to monkeys, rats, and mice and, with some types of spirochæte, to guinea-pigs; rabbits are usually refractory. In monkeys the disease runs much the same course as in man; 2 or 3 days after subcutaneous inoculation with the patient's blood a pyrexial attack occurs, lasting for 3 or 4 days; two, three, or four relapses may occur at intervals of 2 to 8 days, each relapse lasting from 1 to 4 days (Norris *et al.* 1906). After intraperitoneal inoculation of mice the organisms appear in the blood within 24 hours, and persist for 3 to 4 days; they then disappear for several days, after which a relapse may occur; three or four relapses may follow each other, separated by an interval of about 7 days (Novy and Knapp 1906). Sometimes the animals die after 4 or 5 days. As many as 10 to 50 organisms may be present per field during the first infection, but in the relapses only 1 or 2 organisms are seen as a rule. Intraperitoneal inoculation of white rats is followed by the appearance of spirochætes in the blood in about 40 hours; they disappear about 2 days later. The spirochætes are found not only in the blood, but in all the organs of the body. Infection is never fatal. Novy and Knapp (1906) state that rats do not relapse, though Bohls and Irons (1942) deny this. Guinea-pigs are relatively insusceptible to most strains, but are said to be easily infected with *Trep. hispanicum* and *Trep. sogdianum*, and often to die of hæmorrhagic peritonitis after inoculation with *Trep. persicum*. *Trep. usbekistanicum* is said to be uniformly fatal to them (Delpy and Rafyi 1939).

Diagnosis.

Blood should be taken during the pyrexial period and examined either by dark-ground illumination or after staining with Wright, Giemsa or gentian violet (see Beck 1936, Bohls and Irons 1942). If the staining technique is adopted, it is usually wise to make a thick film preparation by stirring 2 or 3 drops of blood on one slide with the corner of another slide till they cover an area about 1 cm. in diameter. Unless they are stained at once, they should be laked in distilled water or 1 per cent. acetic acid before staining. Thin films to be stained by Giemsa or gentian violet should be previously fixed for 3 minutes in methyl alcohol. Microscopically, the spirochætes are usually seen in large numbers in blood taken during the febrile period, but later they may be scarce and are often coiled up or clumped together.

For confirmation of the microscopical result, or for enrichment if this is negative, 1–2 ml. of fresh whole blood, or of clot ground up in saline, should be injected intraperitoneally into a susceptible animal—preferably the mouse. Spirochætes may not become visible microscopically in the mouse's blood for 2 to 3 days, and sometimes for much longer. A drop or two of blood from the tail should be examined every day for at least a fortnight from the second day onwards, if the animal does not die of the infection.

Spirochætes may sometimes be demonstrated by animal inoculation in the blood of patients for weeks after the primary attack. According to Chung (1938) the urine and prostatic fluid are sometimes infective. In making rodent surveys, the spleen, heart, or brain tissue should be ground up in saline and inoculated subcutaneously into white mice; the blood is then examined daily for spirochætes in the usual way. It is worth noting that the Wassermann reaction may be positive in relapsing fever. A method of diagnosis by serological means has been described (Stein 1944).

Prophylaxis and Treatment.

Little can be done by the individual to protect himself against infection in an endemic area beyond avoiding lice or ticks. Inoculation of living cultures, or sometimes of cultures killed by heating to 60° C. for 30 minutes, is said to give rise to the production of lysins. Persons so vaccinated may apparently resist infection with small doses of living spirochætes (Aristowsky and Wainstein 1929*a*, *b*). Whether vaccination is likely to be of value in practice it is as yet impossible to say. Like most spirochætal diseases, relapsing fever is readily cut short by injection of salvarsan or neoarsphenamine. A single suitable dose often suffices to cure the disease and prevent relapses (Wenyon 1926). However, just as " serum-fast " variants may develop during the course of the disease, so " arsenic-fast " strains may be encountered, which are little affected by salvarsan. Infections with such strains may be treated by sodium potassium bismuth tartrate (Todd 1930). The serum of convalescent patients may be used therapeutically, but little information is available about the value of this method of treatment. More recently, *Trep. recurrentis* has been reported to be susceptible to penicillin (Lourie and Collier 1943), so that in future resistant cases of the disease may perhaps prove amenable to treatment with this substance.

AVIAN SPIROCHÆTOSIS

In 1891 Sakharoff described a disease of geese that appeared every year in certain stations on the Transcaucasian railway, and resulted in a high mortality —80 per cent. Examination of the blood revealed the presence of spirochætes closely resembling those of human relapsing fever. Clinically, the infected goose went off its feed, remained apathetic in a sitting-down posture, and died of exhaustion after a week or more ; sometimes it developed diarrhœa, and its joints became affected. Post mortem, there was fatty degeneration of the heart and liver ; the liver moreover showed miliary yellowish granules of caseous consistency ; the spleen was soft and friable. During life actively motile spirochætes were found in fairly large numbers in the blood at the beginning of the disease ; later they collected into ray-forms, and finally into tangled ball-like masses. Before death they disappeared from the blood and at post mortem they could be found neither in the blood nor in the organs. Subcutaneous inoculation of infected blood reproduced the disease in normal geese after an incubation period of 4 or 5 days.

Working with the spirochæte of goose septicæmia—*Treponema anserinum*—Gabritschewsky (1898) found that if the blood serum of a goose, which had recovered naturally from the disease, was mixed with the blood of an infected goose, the spirochætes were killed in a few minutes at 37° C. ; if no immune serum was added, they lived for 18 hours. When the serum was heated to 60° C., its bactericidal power was destroyed completely. Further experiments showed that, while blood serum from a peripheral vein or from the right side of the heart killed the spirochætes in half an hour or less, juice sucked from the viscera—spleen, liver, kidney, and bone-marrow—did not kill them for 2½ to 9 hours. He also found that, in infected geese, spirochætes could still be demonstrated in the internal organs after they had disappeared from the blood. He concluded therefore that the spirochætes were destroyed in the blood by the spirochætocidins, but remained latent in the viscera where the antibodies appeared to be less concentrated. This conception of immunity is similar to that reached in the case of human relapsing fever.

As well as the spirochætocidins, there are also lysins present in the blood, and agglutinins, which are responsible for the clumping of the organisms. Phagocytosis occurs, apparently as a secondary phenomenon, after the death or immobilization of the spirochætes, though according to Himmelweit (1933) it plays an active part in the destruction of the living organisms. Gabritschewsky further showed that naturally recovered geese remained immune to further infection, as long as bactericidins were present in the blood. Antispirochætal serum, prepared by the inoculation of horses, had marked protective properties when injected into geese simultaneously with, or 24 hours after, injection of spirochætal blood; but once the organisms had appeared in the blood stream, it had no effect. That is to say, the serum had prophylactic, but not therapeutic properties. By injection of normal geese with a single dose of immune serum, followed on the next day by injection of spirochætal blood, he succeeded—by active immunization under cover of a passive immunity—in rendering the birds highly resistant to the disease. This method he recommended for combating natural outbreaks of the disease.

More widespread than the disease in geese is **spirochætosis of fowls,** which was first described by Marchoux and Salimbeni (1903) in Brazil, and later by Balfour (1908) in the Sudan. The symptoms and general course of the disease appear to be much the same as in geese.

The disease can be transmitted by subcutaneous, intramuscular, or intraperitoneal injection of hens with infected blood; the bird develops diarrhœa, the temperature rises to 43° C., and spirochætes appear in the blood in 24 hours. The temperature falls after 3 or 4 days to 40° or 41° C., but the organisms continue to increase in the blood. Later, however, they become aggregated into groups, their movements become slower, and they form figures of 0 and 8. At this stage the bird dies, though sometimes a chronic disease develops, lasting for about a fortnight, and followed by death. The disease can be transmitted to fowls, geese, ducks, guinea-fowls, and sparrows; pigeons are fairly refractory, monkeys and guinea-pigs completely so (Marchoux and Salimbeni 1903). Neither in the goose nor in the fowl do relapses occur; the bird either dies or recovers completely.

The natural disease is spread by ticks. In Brazil *Argas miniatus* was found to be responsible; in the old world *Argas persicus* is the tick that has been chiefly incriminated. Marchoux and Salimbeni (1903) found that ticks might remain infective for 5 months after biting a diseased fowl. According to Hindle (1912) ticks may transmit the infection to their progeny, and these again to the next generation, without having had an infective feed in the meantime.

The disease occurs in **ducks** as well as in fowls and geese. It is probable that the causative organism is the same in each species; its proper name therefore is *Treponema anserinum* (Wenyon 1926). Those anxious for further information on avian spirochætosis are referred to the monograph of Knowles, Gupta, and Basu (1932) who, besides giving a bibliographical review of the subject, have made a number of observations themselves, particularly on the mechanism by which immunity develops.

Blood Spirochætoses in other Animals.

Cattle suffer from a spirochætosis caused by *Treponema theileri*; infection is conveyed, at least in South Africa, by the tick *Margaropus decoloratus*. A similar disease in horses and in sheep, probably due to the same organism, *Trep. theileri*, has also been reported by Theiler in South Africa. Spirochætes of the relapsing fever type have been observed in the blood of elephants, camels, antelopes, monkeys, and some other mammals (Wenyon 1926).

VINCENT'S ANGINA AND CERTAIN RELATED INFECTIONS

There are certain necrotic and gangrenous infective processes in human beings, such as ulcero-membranous gingivitis, hospital gangrene, noma, fœtid bronchitis, and gangrenous laryngitis, in which spirochætes have frequently been demonstrated. Of these, one of the chief is the so-called spirillum described by Vincent (1896, 1899), now known as *Treponema vincenti* (see Chapter 38). It is not clear whether Vincent's spirillum is responsible for the necrotic lesions in which it is found, or whether it is a mere secondary invader. The fact that it is often present in scrapings of the gingivo-dental fold in apparently healthy mouths has led many observers to doubt its ætiological rôle in the inflammatory diseases just mentioned. Black (1938), for example, found Vincent's spirillum in 60 per cent. of children under 12 years of age, fusiform bacilli in 94 per cent., and both together in 18–63 per cent. according to age. He regards these organisms as members of the normal flora of the mouth, which multiply when conditions are favourable, but which are without pathogenic action. Recent work (see p. 1897) has shown that some of the gingivo-stomatitis of children is a primary herpetic infection; but there is nevertheless a group of diseases characterized by ulceration of the gums and throat, in which no virus has so far been demonstrated, but in which Vincent's spirilla are abundant. The organisms are often found associated with a characteristic fusiform bacillus, likewise described by Vincent (1896, see Chapter 18). It has been suggested (Tunnicliff 1906) that *Trep. vincenti* and the fusiform bacillus represent two phases of the same organism; but the balance of evidence is definitely against this view. Since we know that strict anaerobes are unable to grow in healthy tissue, the finding of large numbers of spirochætes and fusiform bacilli in swabs taken from the ulcerated gums is an index of the presence of necrotic tissue or of non-living matter in the mouth. In fact, the disease is often associated with septic teeth, salivary calculi, or an erupting third molar. Why the disease becomes almost epidemic in war-time is still unknown. Shortage of ascorbic acid or of nicotinic acid in the diet has been suggested, but the evidence is not convincing. Infection from one person to another in camps or communal feeding centres probably plays some part. Arsenic has been used in treatment, but its value is doubtful. Measures directed to cleaning up the mouth, the removal of dead tissue, and of tartar on the teeth, and the use of hydrogen peroxide washes and zinc peroxide pastes seem to be more effective. Penicillin is reported to be of benefit.

Treponemata have also been isolated from the sputum of tuberculous patients (Bezançon and Etchegoin 1926), and from other bronchial and pulmonary lesions (Bacigalupo 1928); but we know nothing with regard to the relation of these organisms to each other or to *Trep. vincenti.*

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CHAPTER 81

SYPHILIS, RABBIT SYPHILIS, AND YAWS

SYPHILIS

THE origin of syphilis is not definitely known, but its presence in Europe is said to date from the end of the fifteenth century, when it was brought to Spain by Columbus's sailors returning from the New World. The disease spread rapidly over Europe, and was at first endowed with the malignancy that is characteristic of most infective diseases on first reaching virgin soil. Now, after centuries of endemic prevalence, the disease is much milder; this is probably due in part to better and more rapid treatment, and in part to the development of a racial immunity, similar to that which appears to have developed against tuberculosis.

Edwin Klebs in 1875–77 was apparently the first to see spirochætal bodies in syphilitic material and to transmit the disease to monkeys (see A. C. Klebs 1932). Haensell in 1881 produced a local lesion in rabbits by inoculation of the eye. Metchnikoff and Roux (1903, 1904*a*, *b*, 1905) transmitted the disease to apes, and found that chimpanzees developed not only primary but also secondary lesions following inoculation with human syphilitic material. In 1905 Schaudinn and Hoffmann discovered the causative organism of the disease. In chancres and in the inguinal glands of syphilitic patients they demonstrated a spirochæte, now known as *Treponema pallidum*, which occurred both on the surface and in the depth of the tissue. They stained it by a modified Giemsa stain, and described its characteristic morphology and movements. In chancres it was frequently accompanied by another spirochæte—*Trep. refringens*—which was broader, less regular, and more refractile. But *Trep. refringens* was sometimes found in non-syphilitic lesions, such as gonorrhœal papillomata, whereas *Trep. pallidum* was never found except in syphilis. Though Schaudinn and Hoffmann did not decide whether *pallidum* and *refringens* were different species, it has since been shown that it is *Trep. pallidum* alone that is responsible for the causation of syphilis. (For further history see Schuberg and Schlossberger 1930, Stokes 1931, Klebs 1932.)

Bacteriology.

Syphilis is transmitted chiefly by direct, but sometimes by indirect contact. *Treponema pallidum* has the power of gaining entrance to the body through minute lesions of the skin or mucous membranes. It is, however, a very strict parasite, and its life outside the animal body is short; if this were not so, syphilis of non-venereal origin would probably be very much more common than it is. Infection may likewise be transmitted from mother to child, causing the congenital type of syphilis; this results from the passage of spirochætes through the placenta. Except in congenital syphilis, in which the disease is generalized from the

start, infection is rendered evident by the development of a primary lesion or chancre. This appears within a month of infection, and is accompanied by enlargement of the focal lymphatic glands. From 6 to 12 weeks after the appearance of the primary chancre, the secondary stage of the disease sets in; this is marked by constitutional symptoms, cutaneous lesions, enlargement of the lymph glands, and often affections of the bones, joints, eyes, and other organs. The secondary passes over insensibly into the tertiary stage, which may persist for years; this is characterized by the development of ulcerating necrotic lesions of the skin and mucous membranes and by gummata of the internal organs. Years after the contraction of the disease disorders of the nervous system may appear, such as tabes dorsalis and general paralysis; these are sometimes referred to as quaternary or parasyphilitic affections.

Up to the commencement of the secondary stage, syphilis is clinically a localized disease; bacteriologically, however, it appears probable that infection becomes generalized soon after infection. Kolle and Evers (1926*b*) infected rabbits by cutaneous or subcutaneous inoculation into the scrotum with syphilitic material, removed the inguinal glands after varying periods, and injected these into fresh animals. By this means they found that the glands were infective within 30 minutes of the scrotal inoculation. Working with guinea-pigs, they were able to show that the spirochætes reached the focal glands within 5 minutes of cutaneous inoculation of the scrotum. In apes the time elapsing between infection and invasion of the glands is probably longer; Metchnikoff and Roux (1905), for example, found that chimpanzees anointed locally with calomel ointment, 1 to 2 hours after cutaneous inoculation, never developed syphilis. Taking this experimental evidence in conjunction with the fact that local disinfective measures in human beings are comparatively valueless unless practised within an hour or two after exposure to infection, we may conclude that the spirochætes rapidly invade the tissues, even though they give no clinical sign of their presence. During the primary stage of the disease, they are found in the local chancre, and can sometimes be demonstrated in the blood. Thus, Uhlenhuth and Mulzer (1913) drew off the blood of patients with primary and secondary syphilis, defibrinated it, and injected it into the testicles and scrotum of rabbits; the whole operation was completed within 10 minutes. Syphilis developed in 67 per cent. of the animals inoculated from patients with primary, and in 70 per cent. of those inoculated from patients with secondary syphilis. Spirochætes are present in all the secondary lesions, and may be excreted in the semen (Uhlenhuth and Mulzer 1913). In the tertiary lesions such as gummata they are demonstrable, but only in small numbers; their virulence, however, appears to be maintained. Noguchi and Moore (1913) found them in the brain of patients dying of general paralysis; they were seen in all the layers of the cortex with the exception of the outer or neuroglial layer. In congenital syphilis spirochætes are distributed in large numbers throughout the viscera, particularly the liver, lungs, spleen, and suprarenals. Both in congenital and acquired syphilis the organisms may remain latent for long periods of time without giving rise to any clinical manifestations of disease.

Immunity to Syphilis.

Immunity to syphilis resembles in many respects immunity to tuberculosis; that is to say, it is an infection-immunity. So long as infection persists, and so long as living spirochætes are present in the tissues, the infected person is rela-

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tively resistant to further infection. It is possible for a patient to develop two primary chancres, provided the second infection occurs before the development of the first chancre; but once a primary chancre has developed, further infections almost invariably fail to give rise to a primary lesion. There is evidence to suggest, however, that this resistance to superinfection is more apparent than real, in the bacteriological sense. Though the second infection gives rise to no obvious symptoms of disease, the spirochætes nevertheless appear to gain access to the body and to give rise to a latent infection of the tissues.

Experiments indicate that immunity to superinfection is limited by various factors. Truffi (1910), for example, was able in some rabbits to produce a chancre by inoculation made as late as 12 days after the primary chancre had developed. Kolle and Schlossberger (1926) found that, whereas rabbits reinfected with the same strain of spirochæte did not develop a second primary lesion, those infected with a heterologous strain frequently did so. And even when no second primary lesion developed, it was shown by inoculation experiments made with the lymphatic glands that the organisms had gained access to the tissues; by the use of cross-protection experiments in two sets of rabbits, each of which was immunized against 1 strain only, it was shown that the homologous and the heterologous strains were present side by side in the same glands. According to Uhlenhuth and Grossmann (1928) the age and the virulence of the first infecting strain are of importance; infection with a weakly virulent strain may not protect against subsequent infection with a more virulent strain. Working with apes Neisser (1906) succeeded in reinfecting 9 animals at periods varying from 44 to 301 days after the first inoculation. These and other experiments indicate that, though as a rule an infected animal is resistant to superinfection, in the sense that it does not develop clinical symptoms, it may on occasion prove susceptible; and, even when it does not, it may yet allow the penetration of the organisms into its tissues; its immunity to superinfection is largely a symptom-immunity.

Other experiments support the view that infection may occur without the production of symptoms.

Kolle and Evers (1926*a*) found, for example, that 10 out of every 100 rabbits inoculated into the scrotum failed to develop a primary chancre or to show glandular swelling. These animals were nevertheless infected; the popliteal glands from 12 of these animals were inoculated into fresh animals from 82 to 199 days after infection, and in every instance they produced infection. In such animals the organisms generally lie latent in the glands, but occasionally they become active and give rise to lesions several months later. The same authors found that if rabbits containing a deposit of bismuth in their tissues were inoculated with syphilitic material, they developed no lesions whatever; but if the bismuth was later removed by surgical means, typical syphilitic lesions developed 7 or 8 weeks later. It would appear that the effect of the bismuth was not to prevent infection but to suppress the development of lesions; the spirochætes gained access to the tissues, but remained latent till the bismuth was removed. In some animals, such as mice, inoculation with syphilitic material is usually without result; no lesions develop. Yet by inoculation experiments on rabbits it has been shown that spirochætes are present in their tissues—glands, spleen, and brain—for months after infection (Kolle and Schlossberger 1928). There is evidence that the organisms actually multiply in the tissues of the mouse; thus for 6 to 8 weeks after infection the organs frequently prove negative, but after that time, and apparently for the remainder of the mouse's life, they are positive. Yet in spite of this development in the tissues, the organisms never give rise to symptoms of disease.

Whether a true immunity both to clinical disease and to infection ever develops in rabbits is not clear. There is evidence that it may, but only in animals that have been infected for a long time.

Brown and Pearce (1921) treated rabbits with arsphenamine shortly after the development of the primary chancre, and 5 days later reinoculated them with the same strain as was originally used. Nearly all the treated animals developed chancres, whereas the untreated controls did not. This indicates that animals treated in the early stage of the disease can be rendered fully susceptible again. Chesney and Kemp (1925) obtained evidence suggesting that if treatment was begun early the animals almost always became susceptible to a second infection, whereas if it was begun late they generally proved refractory to a second infection. Further experiments (Chesney and Kemp 1926, Chesney, Halley, and Kemp 1927), however, seemed to show that the immunity to reinfection of rabbits treated late in the disease was not as complete as was originally supposed. Kolle and Prigge (1927) treated their rabbits with neosalvarsan in the late stage of the disease —75 to 250 days after infection. Reinfection gave rise to no symptoms, but inoculation experiments showed that the spirochætes had gained access to the tissues; in non-reinfected controls the tissues proved sterile. This indicates that animals treated in the late stage of the disease, and cured of infection, remain clinically immune; they develop no symptoms of disease on reinfection, but they are not sufficiently resistant to prevent the organisms from gaining access to the tissues. Uhlenhuth and Grossmann (1928) treated their rabbits with neosalvarsan in the late stage of the disease—107 to 506 days after infection. Reinfection with the homologous strain performed 3 to 10 months after treatment gave rise to no symptoms; and in only 4 out of 11 rabbits were spirochætes demonstrated in the tissues. This indicates that a certain proportion of rabbits treated in the late stage of the disease remain both clinically and bacteriologically resistant to fresh infection. Similar results have been recorded by Breinl (1935).

Resuming the general findings, it may be said that untreated rabbits in the late stage of syphilis usually show a chancre-immunity to the homologous strain, even though there is no general immunity. That is to say, their immunity is sufficient to prevent the formation of a characteristic local lesion on reinfection, but is insufficient to prevent the development of the inoculated organisms in the tissues. Syphilitic rabbits treated with salvarsan within 45 days after infection only exceptionally develop a chancre-immunity; those treated 45–90 days after infection frequently do so, while those treated later than 90 days after infection almost invariably do so. The real divergence of opinion between different workers concerns the existence of a general spirochætocidal immunity in rabbits treated in the late stage of the disease. Uhlenhuth and Grossmann (1928) are convinced that such an immunity does in fact develop, while Kolle and Prigge (1927, 1929, 1934) are as equally convinced that it does not.

It would perhaps be sententious to suggest that the truth lies somewhere between the two, and that rabbits treated in the late stage of the disease are more immune than those treated in the early stage, though not always sufficiently immune to withstand completely invasion by fresh organisms. The discrepancy between the results of the two main protagonists is probably more apparent than real. Indeed, in a more recent publication Grossmann (1933) himself brings evidence to show that, though a general immunity does develop as the result of long-standing infection, it is of comparatively short duration. (See also Chapter 51.)

How closely the immunity of human beings to syphilis resembles the immunity of rabbits is not known. Judging from clinical observations it would appear that the two are not dissimilar. There is no satisfactory evidence that a spon-

taneous cure occurs either in rabbits or in man; once the organisms have given rise to active symptoms of disease, they remain latent in the tissues for an indefinite period. Every now and again they may become active, or they may remain permanently in the latent condition. Both rabbits and man can be cured if they are treated by salvarsan in the early stage of the disease; in the later stages a complete cure is very difficult to attain. Even though in man the Wassermann reaction may be rendered temporarily negative, it frequently becomes positive again after treatment is stopped.

It is very important to know whether patients with latent syphilis are infective, but unfortunately our knowledge on the subject is scanty. Animal experiments suggest that female rabbits suffering from latent syphilis may transmit the disease to healthy bucks; but apparently no primary lesions develop as in the usual type of rabbit syphilis; the spirochætes can, however, be demonstrated in the regional glands (Kolle 1928, Albrecht 1930). Kolle thinks that syphilis in human beings is often contracted in this way. Many persons are discovered in the course of routine examination to be suffering from syphilis who never suspected it, and who never showed evidence of a primary lesion. In Kolle's view such persons may have been infected by latent carriers of the disease, and have themselves acquired a latent infection without developing the usual primary lesions.

Another view has been put forward by Levaditi (Levaditi *et al.* 1928) and his collaborators. Starting from the observation that spirochætes can only rarely be found microscopically in the focal glands of rabbits infected by the scrotal route, even though these glands are almost invariably infective to fresh animals, he supposes that the virus of syphilis undergoes a development cycle of which *Treponema pallidum* is only one of the stages. In certain rabbit lesions, particularly those that were undergoing retrogression, he observed involution forms of the spirochæte, amongst which were minute granules only just visible by the microscope. He supposes that the spirochætes pass into a finely granular stage; that these granules are more resistant to arsenic and its compounds; and that they are responsible for latent infections in syphilis, and possibly for certain cases of tabes and general paralysis in which spirochætes cannot be demonstrated. Similar hypotheses have been put forward by other workers, but the evidence in their favour is unsatisfactory. Van Haelst (1933), who has carefully studied the question, finds that the successful transmission of infection to rabbits is to a considerable extent determined by dosage. The inability to demonstrate spirochætes in tabes may quite possibly be due to the very small number present in the tissues. If there is an ultramicroscopic phase of the organism, then filtrates of virulent organs should prove infective; but according to Levaditi's own findings they do not.

Diagnosis.

In the primary stage of the disease, *Trep. pallidum* may be demonstrated in the chancre. The superficial part of the lesion should be cleansed by gentle swabbing with saline, and exudate should be drawn from the base of the chancre for examination; this may conveniently be obtained by applying a small suction-cup with the usual rubber-ball attachment. The serous exudate should be examined under dark-ground illumination. The morphology of *Trep. pallidum* is described on p. 915. The eye-piece micrometer devised by Barnard (1923), with which an approximate measurement of the spirals can be made with ease and rapidity, forms a most useful aid in the identification of this organism. According to Goldsworthy and Ward (1942), dark-ground illumination may be replaced by a simple stain, such as Victoria blue 4R, or carbol gentian violet in conjunction with the Fontana mordant.

It cannot be too strongly emphasized that the identification of *Trep. pallidum* in a primary chancre requires the expert knowledge which comes only with long experience. It depends on the recognition of fine differences in morphology, without the assistance of characteristic differences in staining reaction, or of confirmatory tests on pure cultures. It is fortunate that the primary penile chancre provides the large majority of cases which the bacteriologist is called on to examine, since the spirochætal flora in such lesions is usually not copious, and consists mainly of species, such as *Trep. refringens*, which are readily differentiated from *Trep. pallidum*. Chancres in other situations, such as the lip, or primary genital chancres in the female, are far more likely to show a complex spirochætal flora, including species closely resembling *Trep. pallidum* ; and, in such cases, the greatest caution should be observed in basing a diagnosis of syphilis on a microscopical examination alone. It happens that a penile chancre usually leads to a suspicion of syphilis at an earlier stage than a genital chancre in the female, or an extra-genital chancre in either sex ; so that the latter lesions tend to be overlooked in the early stages, and, by the time they have attracted attention, the infection has usually reached the stage at which the Wassermann reaction is positive, thus providing a most valuable check on the result of the direct microscopical examination.

In the early stage of the disease it is possible to demonstrate spirochætes in excised lymph glands by inoculating them intratesticularly into rabbits, and following the progress of infection in these animals by the Kahn test and by dark-ground examination of the emulsified testicle. Negative results, however, are often obtained in treated cases and even in old untreated cases (Lake and Bryant 1930). The method is of academic rather than of practical interest.

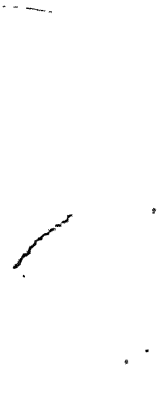


FIG. 296.—*Treponema pallidum.*
In material scraped from a hard chancre. Fontana. (× 1000).

Diagnostic Serum Reactions.

The serum reactions employed in the diagnosis of syphilis have, on account of their clinical importance, been submitted to detailed and extensive study, out of all proportion to their general immunological interest. The literature is correspondingly copious and diffuse, and it is quite impossible to review it here, or to attempt any discussion of the various points at issue. We can give only a brief description of some of the available tests and a short discussion of their interpretation.

The Wassermann Test.—Wassermann, Neisser and Bruck (1906), and Wassermann, Neisser, Bruck, and Schucht (1906) described a complement-fixation reaction, using as an antigen a watery extract of the liver of a syphilitic fœtus. This test

was found to give positive results in persons suffering from syphilis, and negative results in normal controls. The syphilitic liver was selected as a convenient source of *Trep. pallidum*, which had not then been cultivated ; and the reaction was at first supposed to be specific in the bacteriological sense. It was soon found, however, that this was not the case (Marie and Levaditi 1907), since extracts of normal liver gave identical results. Still better results were obtained when watery extracts were replaced by alcoholic extracts of various tissues, and it was shown that the reacting substance was contained in the lecithin fraction of the lipoids, which is soluble in alcohol but insoluble in acetone (Porges and Meier 1908). This so-called " antigen " is rendered more sensitive, as regards its reaction with syphilitic sera, by the addition of an alcoholic solution of cholesterol (Browning and Mackenzie 1924). The antigen most commonly employed at the present time is an alcoholic extract of heart-muscle from the ox, calf, or man, from which the acetone-soluble fraction has been removed. To this alcoholic extract is added an alcoholic solution of cholesterol, so that the cholesterol content is 0·3–0·4 per cent. ; and the combined alcoholic solution is suitably diluted with saline. With this " antigen " a complement-fixation test is carried out in the usual way, and with the usual controls. The Wassermann reaction is, therefore, in no way specific in the bacteriological sense ; its basis is essentially empirical. It has been found in practice to be one of the most useful and reliable methods employed as laboratory aids to diagnosis ; but its diagnostic significance depends entirely on an observed correlation with clinical findings, for which there is, as yet, no satisfactory explanation. The methods of performing the test are legion (see Reports 1918*a*, 1924, 1929, 1934, Cumming *et al.* 1935, Vogelsang 1940) and many modifications have been suggested to increase its sensitivity. The principles are, however, in no way different from those outlined in Chapter 7, in connection with complement fixation in general.

Three reagents are concerned in the actual reaction—antigen, patient's serum, and complement. When these have been allowed to react for a suitable period—1 hour at 37° C., or overnight in the ice-box—sensitized red cells are added, and the mixtures are incubated for 1 hour at 37° C. to determine the presence or absence of hæmolysis, indicating he presence or absence of una bsorbed complement. To establish a positive reaction it must be shown (*a*) that the patient's serum alone absorbs no complement, or at most a small fraction of that present in the test mixture ; (*b*) that the same is true of the antigen ; and (*c*) that the mixture of serum and antigen absorbs an amount of complement greatly in excess of the sum of the small amounts absorbed by each of these reagents separately. Thus the essential tubes in carrying out a Wassermann reaction are (1) the antigen control, (2) the serum control, and (3) the test proper. The first control will serve for the whole series of tests carried out on any one day, the second must be put up for each serum tested. It is, however, obviously desirable to obtain a more accurate measure of the strength of the reaction than is possible by such a simple method as this ; and most of the methods in actual use are designed to yield a roughly quantitative result, which will enable the reactions to be graded into strongly positive, positive, weakly positive, doubtful, and negative. We may consider briefly the possible ways in which such results may be obtained, and the lines along which some approach to standardization may be sought.

There are three variables involved in the reaction—antigen, serum, and complement ; clearly, we may obtain quantitative results by holding any two of these constant, and varying the third. Varying the antigen introduces factors which need careful reconsideration in view of the recent advances in our knowledge of the importance of optimal relations in antigen-antibody reactions ; at the present time this method is seldom, if ever, adopted. With the amount of antigen held constant we may vary either the test

serum, or the complement. The latter method is very commonly employed, the serum-antigen mixtures being allowed to react in the presence of 3 and 5 M.H.D., or of 2, 4, 6 and 8 M.H.D. In either case the serum and antigen controls are put up with the smallest amount of complement employed in the test. In this way we grade the strength of a reacting serum in terms of the amount of complement which is fixed when a constant amount of serum (usually 0·5 ml. of a 1 : 5 dilution) is mixed with a constant amount of antigen (0·5 ml. of a suitable dilution), and the same volume of the suitably diluted guinea-pig's serum is added to the mixture.

An alternative method, advocated by several workers, is to hold antigen and complement constant, and vary the amount of patient's serum, testing it in progressive dilutions of, say, 1 : 2·5, 1 : 5, 1 : 10, 1 : 20, 1 : 40, though it is not usual to employ more than three dilutions. This method has the advantage of giving a direct measure of the concentration of the Wassermann-antibody in the serum under test. The serum control is, of course, put up with the largest amount of serum employed in the actual test.

In either case it is necessary to standardize the complement. For this purpose a preliminary titration is carried out, to determine the minimal hæmolytic dose. It is convenient to carry out this titration in duplicate. In the first series one volume of progressive dilutions of guinea-pig serum is added to two volumes of saline—0·5 ml. is usually taken as "one volume" throughout; in the second series the same dilutions of guinea-pig serum are added to one volume of saline and one volume of the antigen to be used in the test. After 1 hour's incubation at 37° C., or a longer time in the ice-chest, one volume of a 5 per cent. suspension of red cells and one volume of a hæmolytic serum, so diluted as to contain at least 5 M.H.D. of hæmolysin, are added to each tube; and both series are then incubated for 1 hour at 37° C. In this way we are able to determine the M.H.D. of complement (guinea-pig serum), before and after it has been allowed to react with the antigen. This obviates the necessity for any further antigen control, and enables us to detect any excessive anti-complementary action of the antigen, or any tendency of a particular specimen of guinea-pig's serum to react with the antigen alone. If such a test shows any marked difference in the M.H.D. in the two series, it may be taken that either the complement or the antigen is unsuitable, and must be replaced by a more satisfactory sample. In the test itself it is usual to employ 3 M.H.D. of complement, if this reagent is to be held constant. The use of dried complement, or of complement preserved in acid saline (Richardson 1941), adds considerably to the convenience of the test.

Precipitation Reactions, including the Kahn Test.—From our knowledge of the antigen-antibody reactions in general, we should expect that any reaction which gives complement fixation under a particular set of conditions will give visible precipitation if the conditions are properly adjusted. So far as the lipoid antigens and the serum antibodies involved in the Wassermann reaction are concerned, this is the case. The various methods for applying the precipitation test to the diagnosis of syphilis are scarcely less numerous than the modifications of the Wassermann reaction (Michaelis 1907, Meinicke 1907, Sachs and Georgi 1918, Vernes 1918, Dreyer and Ward 1921, Kahn 1922*a*, *b*, 1928, Report 1923, Report 1929, 1939, 1942, Hinton 1932, Kline 1932, Eagle 1932, Cumming *et al.* 1935, Rytz 1936, Laughlen 1935, 1938, Rappaport and Eichhorn 1943). For a general description of these tests, the monograph by Vogelsang (1940) may be consulted. A precipitation test possesses certain obvious advantages as compared with a complement-fixation reaction, for routine diagnostic purposes. It necessitates the use of two reagents only—serum and antigen. It is more easily standardized, and it is simpler to perform. The Kahn test, in particular, has been widely used during recent years.

The antigen employed in this test (Kahn 1928) is of the same general type as that used in the Wassermann test; but the preliminary extraction of the dried heart muscle, to remove the residual lipoid components, is carried out with ether instead of with acetone. The ether-extracted heart muscle is then extracted with alcohol, and cholesterol is added to the alcoholic extract. In preparing the antigen for use in the test, saline is added until an opalescent colloidal suspension is obtained, which disperses to give a clear solution on the addition of further saline, or serum. When the concentrations of the reagents are correctly adjusted, and flocculation is assisted by shaking, such an antigen, in the presence of a syphilitic serum, yields an almost immediate precipitate, while in the presence of a normal serum the solution remains clear and translucent. In the standard test three tubes are used. The amount of serum is kept constant (0·15 ml.), and the amount of antigen is varied (0·05 ml., 0·025 ml. and 0·0125 ml.). The tubes are shaken for 3 minutes, 1 ml. of saline is added to the first tube, 0·5 ml. to the second and third, and the results are read. In a weak reaction precipitation appears only in the third tube, with perhaps a trace of opalescence in the second; that is, with a serum which contains little "antibody," precipitation is inhibited by excess of antigen.

More recently, Kahn (1940*a*, *b*) has introduced a "verification test," in which the reaction is observed at both 1° C. and 37° C. It is based on the observation that reactions due to syphilitic antibody are generally stronger at 37° C. than at room temperature, and that those due to non-syphilitic antibody are generally stronger at 1° C. than at 37° C. The value of this test is still under discussion (see Chargin and Rein 1941).

The Interpretation of the Diagnostic Serum Reactions.—This is a problem that requires the closest co-operation between the clinician and the clinical pathologist, and its full discussion is beyond the scope of this book. It is considered at length in many of the reports and papers referred to above. For our present purpose we need consider only the results obtained in the most recent systematic surveys.

As a preliminary to any such discussion it is necessary to note the methods employed in recording and reporting results. The tests most frequently used do not permit a reading in terms of any titre, dilution or ratio, and their results cannot therefore be recorded numerically. The most common method of recording is to use an arbitrary combination of plus and minus signs. A strongly positive reaction may, for instance, be recorded as ++++, a less strong reaction as +++, a moderate reaction as ++, a positive but weak reaction as +, a doubtful reaction as +−, or −+, and a frankly negative reaction as −. Sometimes other conventions are used, the plus and minus signs being combined differently, or the strongest reaction being awarded only two, or three, plus signs. Such a notation has nothing to recommend it. The conventional signs used by one pathologist differ from those used by another, and confusion results. Alternatively, words may be used instead of signs; and we then have "strongly positive," "positive," "weakly positive," "doubtful" and "negative" reactions. This is a little better; but there is much to be said for the contention (see Cumming *et al.* 1935) that a division into "positive," "doubtful" and "negative" results gives the clinician all that he can gain, and simplifies the issue. Without some general agreement, these labels will of course be arbitrarily applied, and a proportion of the sera reported to be positive by one test, or one worker, will be returned as doubtful by another. The only way in which this difficulty could be reduced to a minimum would be to standardize the technique, and to issue standardized reagents from central laboratories (see Report 1934).

Taking things as they are, we may inquire what actually happens when sera from syphilitic and non-syphilitic cases are submitted to different kinds of tests. We may take first a trial that was carried out in Copenhagen in 1928, under the

auspices of the Health Organization of the League of Nations. Sera from several hundred persons clinically diagnosed as suffering from syphilis, and several hundred diagnosed as non-syphilitic, were submitted to various serological tests carried out by skilled pathologists from different countries (see Report 1929).

The significant figures taken from this report are tabulated, in a slightly modified form, in Table 178. The various modifications of the Wassermann reaction employed, or the various workers using this test, are designated as W.R.1, W.R.2, etc. The results obtained by the Kahn test are labelled Kahn 1 and Kahn 2. Other forms of precipitation tests are labelled P.1, P.2, etc. The results are recorded as " positive," " doubtful " or " negative." It will be seen that the reports on the clinically syphilitic cases vary rather widely, and that some of the workers recorded rather a high proportion of positive or doubtful reactions among the clinically non-syphilitic cases. Taking the results at their face value, and allowing for the

TABLE 178

RESULTS OF COMPARATIVE TRIALS OF DIFFERENT COMPLEMENT-FIXATION AND PRECIPITATION TESTS.

	Syphilitic			Non-syphilitic.		
	Per cent.			Per cent.		
	Positive.	Doubtful.	Negative.	Positive.	Doubtful.	Negative.
W.R. 1 . .	28·2	15·6	56·2	3·3	8·6	88·2
,, 2 . .	53·7	2·9	43·4	10·7	1·6	87·7
,, 3 . .	41·8	15·5	42·6	—	2·8	97·2
,, 4 . .	52·8	12·9	34·3	6·7	8·3	85·0
,, 5 . .	51·9	13·0	35·1	5·5	9·2	85·3
,, 6 . .	43·9	11·2	44·9	1·4	5·8	92·8
,, 7 . .	38·8	11·4	49·8	—	3·0	97·0
Kahn 1 . .	58·6	5·4	36·0	0·7	1·4	97·9
,, 2 . .	61·1	6·6	32·3	—	1·2	98·8
P. 1 . . .	49·0	7 6	43·4	2·1	3·0	94·9
,, 2 . . .	63·5	9·0	27·5	0·2	2·3	97·5
,, 3 . . .	53·5	14·0	32·5	0·5	8·8	90·7
,, 4 . . .	43·0	4·7	52·3	—	0·2	99·8
,, 5 . . .	52·5	4·3	43·2	—	0·2	99·8
,, 6 . . .	51·2	15·1	33·7	1·4	8·1	90·5
,, 7 . . .	40·1	14·3	45·6	0·6	10·2	89·3

fact that many of the clinically syphilitic cases were clearly not in a stage in which they would give a positive serum reaction, WR.3 with 42·6 per cent. of negatives among the clinically syphilitic, and 97·2 per cent. among the non-syphilitic has given satisfactory results. Both series of Kahn tests do better than this, and so does P. 2.

The variability of the results with sera from clinically syphilitic patients, and the relatively high proportion of negative results, are not surprising; because all stages and types of syphilis were included, and it is well known that the proportion of positive reactions varies widely in different stages and types of the disease. Boas, for instance (see Reports 1918*b*, 1919), records 59 per cent. of positive results in primary syphilis, 90 per cent. in secondary syphilis, 84 per cent. in tertiary syphilis, 72 per cent. in tabes, and 99·3 per cent. in general paralysis of the insane. It is in the type of case that shows a relatively low proportion of positive reactions,

and a relatively high proportion of doubtful reactions, that most discrepancies will occur.

A report by the Public Health Service of the United States (see Cumming *et al.* 1935) gives valuable figures.

Table 179 has been constructed from the data contained in this report and records the percentage of positive results obtained by various tests, and by various workers, in the examination of sera from syphilitic patients in various stages of the disease, and from normal persons showing no evidence of syphilis. The notation used is the same as in Table 178; but it should be noted that Kahn 2 and Kahn 3 refer to modifications of the standard Kahn test, and that the W.R.1, P. 1, etc. of one table are not identical with the W.R. 1 and P. 1 of the other.

TABLE 179

RESULTS OF COMPARATIVE TRIALS OF DIFFERENT COMPLEMENT-FIXATION AND PRECIPITATION TESTS

	Percentage of Sera reacting positively.			
	Untreated Primary Syphilis.	Untreated Secondary Syphilis.	Late Syphilis. Varying Treatment.	Normal. Non-syphilitic.
W.R. 1	53·7	100	66·6	0·0
,, 2	65·9	100	72·1	0·0
,, 3	82·5	100	86·4	0·7
,, 4	69·8	100	58·0	0·0
Kahn 1	76·7	100	76·9	0·0
,, 2*	82·9	100	84·3	3·3
,, 3*	80·5	100	83·0	0·7
P. 1	72·1	100	82·4	2·0
,, 2	81·0	100	84·5	0·7
,, 3	58·1	98·4	64·5	3·3
,, 4	74·4	100	71·5	0·0
,, 5	72·1	98·5	83·6	1·3
,, 6	70·7	98·4	63·1	0·7

* See text.

It will be noted that the proportion of positive results is, on the average, far higher than in the Copenhagen trial; but this may well be due in large part to the nature of the cases examined. Most of the workers recorded 100 per cent. of positives in the untreated secondary cases; but the percentage of positives recorded in the untreated primary infections ranges from 53·7 per cent. to 82·9 per cent., and in the tertiary cases from 58·0 per cent. to 86·4 per cent. (For a further series of comparative trials, see Parran *et al.* 1937.)

More useful information is obtained by making a comparison, not between syphilitic and normal persons, but between persons suffering from syphilis, and persons suffering from some other disease. Very extensive studies have been made along these lines with results that may be summarized as follows. There is general agreement that yaws, which is closely related to syphilis in its ætiology, responds in much the same way to various serum tests. Cases of leprosy give a lower, but still relatively high, proportion of positive reactions. It has often been stated that patients suffering from malaria tend to give positive reactions; but here there has been a considerable conflict of evidence. The same is true of sleeping sickness. Some observers have recorded a small proportion of positive reactions in tuberculosis,

some in febrile conditions, and some in pregnancy. The trial carried out by the Public Health Service of the United States provides information on several of these points, and Table 180 has been constructed from the relevant data, including all the syphilitic cases, and the normal persons, from Table 179.

TABLE 180

RESULTS OF COMPARATIVE TRIALS OF DIFFERENT COMPLEMENT-FIXATION AND PRECIPITATION TESTS.

	Percentage of Sera giving positive reactions.						
	Syphilis all Stages.	Leprosy.	Malaria.	Tubercu-losis.	Fever.	Pregnancy.	Normal.
W.R. 1 . . .	70·5	44·0	14·3	1·9	2·2	0·0	0·0
,, 2 . . .	75·9	64·0	19·4	2·0	2·2	0·0	0·0
,, 3 . . .	88·2	62·0	20·6	7·5	2·2	3·8	0·7
,, 4 . . .	65·8	42·0	17·1	1·9	0·0	0·0	0·0
Kahn 1 . . .	80·5	60·0	11·4	1·9	0·0	0·0	0·0
,, 2* . . .	86·6	76·0	16·7	7·7	2·2	1·9	3·3
,, 3* . .	85·4	68·0	19·4	1·9	0·0	0·0	0·7
P. 1	84·1	72·0	12·1	1·9	0·0	0·0	2·0
,, 2	86·6	40·0	11·1	5·7	2·3	1·9	0·7
,, 3	69·0	58·0	11·4	5·7	4·3	3·7	3·3
,, 4	76·3	66·0	14·3	1·9	0·0	0·0	0·0
,, 5	84·7	70·0	11·1	7·5	8·9	0·0	1·3
,, 6	69·4	52·0	8·6	0·0	0·0	0·0	0·7

* See text.

It will be noted that the frequency of a positive reaction in leprosy is amply confirmed, that malaria yields an appreciable proportion of positive results, and tuberculosis far less, while the figures for febrile conditions and pregnancy differ but little from the normal. Another point will be noticed. On the whole, though there are exceptions, a test that tends to give a high proportion of positive results with sera from syphilitic patients, tends also to give a relatively high proportion of positive results with sera from those suffering with other diseases (see, for instance, W.R.3, Kahn 2, P.2 and P.5), while a test that gives a low proportion of positives in one case tends to do so in the other (see W.R.4 and P.6). Here, again, the standard Kahn test gives satisfactory results.

It is clear that there is not a great deal to choose between diagnosis by complement fixation and diagnosis by a precipitin reaction, so far as reliability is concerned. By either method there will be a certain range of variation in the results recorded by different workers, unless one standardized technique carried out with standardized reagents is to be enforced. In any case, the onus of interpreting the test must rest with the clinician, when he has made due allowance for the stage of the disease, if the case is one of syphilis, and for the possible or probable existence of one of the infections that may sometimes induce similar changes in the serum. For a discussion of the causes of discrepancies in the results of the Wassermann and Kahn tests, the reader is referred to an article by Berger and Sutherland (1944).

As a general working rule, and excluding cases of yaws and leprosy, it may be stated that, in a suspected case of primary syphilis, in the period between the first appearance of the chancre and the development of the secondary rash, a positive

reaction will confirm the diagnosis, but a negative reaction will by no means contradict it. The latter is particularly true during the first 2 or 3 weeks after the chancre appears. In a case which, on clinical grounds, is regarded as secondary syphilis, a negative reaction tells very strongly against the provisional diagnosis. In such cases the test should always be repeated; and, if the second test is also negative, the diagnosis should be very carefully reconsidered. A persistently negative reaction, lasting through this stage, may be taken as practically excluding syphilitic infection. In tertiary syphilis, with the exception of general paralysis, and perhaps of syphilitic aneurysm, a negative reaction cannot be regarded as excluding the diagnosis, though it may tell definitely against it.

The use of the Wassermann reaction in the control of treatment is too complex to be considered here. Broadly, it may be stated that, in a case which comes under treatment early, and which is efficiently treated, the Wassermann reaction becomes negative and remains so; while in a case which escapes treatment during the early stages, or which is inefficiently treated during this period, it is extremely difficult, if not impossible, to produce a permanently negative Wassermann reaction by treatment administered several years after infection has been contracted (Browning and Mackenzie 1924, Report 1926).

It may be added that the tests used in the examination of the blood serum may also be applied to the cerebrospinal fluid in suspected cases of neuro-syphilis; though certain of the precipitin tests give rather poor results when used for this purpose (see Cumming *et al.* 1935).

Such examinations should, of course, be controlled by examination of the fluid for cells, proteins, type of colloidal-gold curve and so on.

A diagnostic *agglutination test* has been described by Tani (1940), in which the patient's serum is mixed with a suspension of spirochætes prepared by the treatment of syphilitic rabbit's testicle with weak antiformin; but it is too early as yet to say whether it will prove of value in practice.

Prophylaxis and Treatment.

We do not propose to discuss the various hygienic methods that have been advocated for the prevention of syphilis.

Bacteriological methods of prophylaxis do not exist. Animal experiments indicate that it is difficult or impossible to protect animals by vaccination with dead spirochætes against subsequent infection with living organisms (Uhlenhuth and Mulzer 1913, Grossmann 1929). Immune bodies have been demonstrated in the blood of infected animals, but so far the transference of passive immunity has been unsuccessful. Metchnikoff and Roux (1904*a*) obtained some evidence that apes inoculated with a weakly virulent strain of syphilis were protected against subsequent inoculation with a fully virulent strain, but the results were not conclusive. Treatment with salvarsan is successful in curing the disease in the early stages, but once the secondary stage has passed, definitive cure appears to be impossible.

A new method of treatment is now on trial, namely, that of hyperpyrexia or pyrotherapy. *Trep. pallidum* is apparently more susceptible to heat than are the tissue cells, and evidence has been obtained that by raising the body temperature to 41·5°–42° C. it is possible in experimentally infected rabbits to destroy the spirochætes *in vivo* (see Carpenter, Boak, and Warren 1932). The hyperpyrexia may be produced either by simple heat, by short-wave radiation, or in certain other ways. It is too early yet to say what value the method is likely to have in practice, but promising results have been reported (see Simpson 1935). A single treatment cannot be relied upon to kill all

spirochætes, but the method may prove useful in conjunction with chemotherapy (Boak *et al.* 1942).

More recently the introduction of penicillin has led to revolutionary ideas on the treatment of syphilis. A single course of one week's treatment seems to be capable in suitable cases of bringing about complete disappearance of the lesions, but it would be premature at the moment to attempt to evaluate this mode of treatment in terms of permanent cure.

Rabbit Syphilis.

Rabbit syphilis is a naturally occurring venereal disease of rabbits due to the spirochæte *Treponema cuniculi.* The disease was first described by Ross (1912) and the spirochætes were first observed by Bayon (1913). The incidence of the disease in wild rabbits varies considerably; according to some authorities as many as 20–40 per cent. in this country are affected. In hutch rabbits the disease is less common; Adams, Cappell, and McCluskie (1928) noticed it in 14 out of 228 rabbits. The lesions consist of small scaly patches, often slightly eroded and covered with a brownish crust, situated on the genitals or in the perineal region. Sometimes the nostrils or eyelids are affected. The spirochætes are found in large numbers in scrapings from the lesions and in sections; they are confined apparently to the superficial layers. The disease can be reproduced in normal rabbits by inoculation of an infective tissue suspension on to the scarified skin of the genital region; transmission can also be effected by mating, but with less constancy. The incubation period is from 2 to 8 weeks; once established, infection persists for months. In males spontaneous cure usually occurs, but in females the disease is very chronic. Rabbits suffering from rabbit syphilis can be infected with human syphilitic material, indicating that the two diseases are not identical (Noguchi 1922). *Trep. cuniculi* is morphologically indistinguishable from *Trep. pallidum*, though Noguchi (1922) says it is perhaps a trifle thicker and longer than the average *pallidum.* Like *pallidum* it stains rose-red with Giemsa. The disease is readily cured by intravenous injection of salvarsan, or by local application of Unguentum hydrargyri. (For further information on the disease and the properties of the causative organism, see Bessemans 1928, and Bessemans and de Geest 1928.)

YAWS

Yaws is a contagious inoculable disease characterized by the appearance of papules, which generally develop into a fungating, encrusted, granulomatous eruption (Manson 1914). It is widely diffused throughout the tropics, and occurs in its typical form only amongst the dark-skinned races. The causative organism, *Treponema pertenue,* was discovered by Castellani in 1905. It is a spirochæte morphologically indistinguishable from *Trep. pallidum.* Yaws occurs particularly in childhood. The commonest site for the primary lesion is on the lower extremities. The secondary eruption usually appears 2–4 weeks later. The disease is not as a rule of venereal origin. Some observers believe that infection occurs through the skin and is carried by gnats. Patients with yaws give a positive Wassermann reaction. The disease is curable by salvarsan and is amenable to treatment with mercury and potassium iodide.

The main interest of yaws is its relationship to syphilis. Many experienced clinicians are of the opinion that yaws is syphilis modified by race and climate.

There is evidence that yaws protects against syphilis. Parham (1922), for example, never once observed syphilis in the Samoans, in spite of their close contact with Europeans; since nearly all Samoans are stated to have yaws in childhood, he argues that the disease must protect them from syphilis in adult life. Similarly Wilson and Mathis (1930), who in a period of 9 years in Haiti saw 45,000 cases of yaws and 300 cases of syphilis, only once met with an undoubted case of syphilis in a patient who had previously suffered from yaws. Further evidence is necessary, however, to determine the relationship between these two diseases.

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CHAPTER 82

WEIL'S DISEASE AND OTHER LEPTOSPIRAL DISEASES: RAT-BITE FEVER

SINCE the discovery by Japanese workers in 1915 of *Leptospira icterohæmorrhagiæ* as the cause of Weil's disease, an increasing number of infections due to leptospiræ have been recognized in different parts of the world. Many of them differ in their clinical picture from Weil's disease, and have been given distinctive names, such as swamp fever, 7-day fever, autumn fever, and so on. As so often happens, the finding of new species similar to that responsible for a well-known disease has created difficulties in terminology. Are we to use the name by which the disease has been described; or is it better to select an all-embracing term such as Leptospirosis, qualifying this, perhaps, as Walch-Sorgdrager (1939) suggests, by adding the specific name of the causative organism, so that we have Leptospirosis grippotyphosa, Leptospirosis hebdomadis, Leptospirosis autumnalis? Our personal preference is, wherever possible, to retain the original name of the disease, even though it means extending the clinical picture beyond that originally described. When the same name has been given to a disease caused by two different organisms, as for example the autumn fever of Japan and the autumn fever of Australia, it may be wise for the sake of clarity to mention the name of the causative leptospira, e.g., autumn fever of the *autumnalis* or the *pomona* type. If we give way to the modern tendency of calling every disease after the generic name of the causative organism, not only shall we have an increase in the already too abundant and monotonous "*oses*" diseases, such as staphylococcosis, streptococcosis, rickettsiosis and so on, but we shall have to abandon the names of age-long familiarity, such as scarlet fever, diphtheria, dysentery, plague, influenza, and typhus fever.

WEIL'S DISEASE

SYNONYM: *Spirochætosis icterohæmorrhagica*

Weil's disease is a fairly widespread and not uncommon disease, which frequently assumes prominence in war-time. During the American War of Secession, 1861–65, over 70,000 men contracted infectious jaundice, that is between 2·0 and 2·5 per cent. of the total strength (Uhlenhuth and Fromme 1916). In the first world war it was common on the Western front, affecting both German and Allied troops (Uhlenhuth and Fromme 1916, Stokes *et al.* 1917, Martin and Pettit 1919). The disease was reported in Scotland in 1923 amongst the coal-miners of the East Lothian district. It appears to be fairly common in South America. It is frequently met with in Sumatra (Sardjito and Zuelzer 1929), and has been observed in the Andaman Islands (Brown 1928, Taylor and Goyle 1931). It is very common in

Japan, where in 1933 there were as many as 1,636 cases. The United States seems to suffer but little. In Europe, Holland and France appear to have the highest incidence rate, but statistics of the real prevalence of the disease are unobtainable (see Report 1934). In Great Britain it is endemic in sewer workers (Fairley 1934, Alston and Brown 1935, Stuart 1939) and in those engaged in washing fish (Davidson *et al.* 1934, Davidson and Smith 1936, Smith and Davidson 1936).

The incubation period of the disease is 5 to 7 days as a rule, rarely as long as 13 days (Inada 1917). In the first or febrile stage, which lasts for 6 or 7 days, there is high fever, conjunctival congestion, muscular pains, and albuminuria. In the second or icteric stage, which lasts from the 7th to the 13th day, jaundice appears, there is a tendency to hæmorrhage, and death may occur. The third stage, or stage of convalescence, is marked by the gradual subsidence of the jaundice and other symptoms; it may be interrupted, however, by a secondary fever (Inada 1917). The mortality of the disease varies in different epidemics; in Japan it varies from 4·6 to 32 per cent. (Inada *et al.* 1916, Report 1934); on the western front during the war of 1914–18 it was under 6 per cent. amongst the British, and 13 per cent. amongst the German troops (Stokes *et al.* 1917, Uhlenhuth and Fromme 1918); in the Scottish outbreak it was about 25 per cent. (Buchanan 1927).

The disease varies considerably in its symptomatology. In something like 60 per cent. of cases no jaundice occurs (Schüffner 1934); these cases are never fatal. Infection may take the form of a serous meningitis, unaccompanied by the usual symptoms of Weil's disease (for references see Walch-Sorgdrager 1939); this form is likewise seldom fatal.

The disease is due to infection with a spirochæte that was first described in 1915 by Inada and his colleagues in Japan (Inada *et al.* 1916). The usual name for this organism is *Leptospira icterohæmorrhagiæ*, but in Germany it is generally referred to as *Spirochæta icterogenes* (see Chapter 38). A few cases appear to be caused by *Lepto. canicola*—a closely allied species that is normally a pathogenic parasite of the dog (Schüffner 1934).

In man the spirochætes are widely distributed in the body during the 1st week of the disease, and can generally be demonstrated in the blood by guinea-pig injection, and occasionally by microscopical examination. After the 7th to 9th day they leave the blood and appear in the urine, at first in very small numbers, but gradually increasing till during the 3rd and 4th weeks they can be found microscopically by dark-ground illumination in the majority of cases. They persist in the urine for a variable time, but can rarely be found after the end of the 5th week. In patients dying during the febrile stage of the disease spirochætes can be demonstrated microscopically in the kidney, liver, adrenals, spleen, testicle, lymphatic glands, voluntary and cardiac muscle, and arterial walls; less frequently in the lung, pancreas, intestine, nervous system, and skin (Inada *et al.* 1916). If death occurs during the 2nd week or later, the chief organ in which spirochætes are found is the kidney; in the other organs they are less numerous or absent.

Occupational Incidence.—Weil's disease occurs chiefly in damp, badly drained, rat-infested situations. Thus in Japan it was noticed that the disease occurred in coal mines; that only the miners who worked in a particular section of the mines were affected; that this section was under water; and that when the water was pumped out, no more cases occurred (Inada *et al.* 1916). Later, it was noticed that cases never occurred in coal mines when the soil was acid; on the other hand

they were numerous in coal mines on an alkaline soil (Ido *et al.* 1917*a*). Experiments have shown, in this connection, that the organisms are rapidly killed by a weakly acid solution. On the western front in 1914–18 Stokes (Stokes *et al.* 1917) observed that the cases occurred mainly amongst the troops in the trenches; at the base they were very uncommon. Most of the cases, moreover, occurred in small sections of trench that were particularly wet and badly drained. Rats taken from one of these sections were found to contain pathogenic leptospiræ in the kidneys. Similar observations were made in the German Army (Uhlenhuth and Zuelzer 1921). Again in Scotland, Buchanan (1927) observed the disease amongst coal miners in the East Lothian district; pathogenic leptospiræ were found in the roof slime of a mine, and similar leptospiræ, the pathogenicity of which was not determined, were frequent in pit and surface waters; infected rats were likewise found in the mines. In the Andamans, cases are practically confined to adult males engaged in outdoor occupations that necessitate working in water and mud for prolonged periods, such as in the rice fields (Taylor and Goyle 1931). In Holland the majority of the cases are contributed by swimmers, bargemen, fishermen, slaughter-house workers, and persons who by accident or with suicidal intent fall into canals, and are nearly drowned after swallowing large quantities of water (Schüffner 1930, 1934). In this country, as has already been stated, sewer workers and those engaged in washing fish are specially liable to the disease.

One very instructive *water-borne outbreak* is on record (Jorge 1932). It occurred in the north-east quarter of Lisbon in 1931, and involved at least 126 persons in the space of a month. Infection was traced to a particular fountain, the water of which during its passage underground in an open conduit had apparently been contaminated by rats from a neighbouring sewer. The case mortality of this epidemic was 24·6 per cent.

Mode of Transmission.—The mode of transmission of Weil's disease is not known with certainty, but there is strong reason to believe that infection takes place directly or indirectly by contamination of the abraded skin or of a mucous membrane, such as that of the eye, nose, or mouth, with rats' urine. The occupational incidence, which has just been referred to, affords striking evidence in favour of this conclusion, since contact with presumably contaminated water or sewage seems to be common to all types of persons attacked. Further evidence is provided by consideration of the frequency with which leptospiræ are found in apparently normal rats. Occasional cases follow an actual rat bite (see Alston and Brown 1937).

Infection of Rats.—It has been established by numerous workers that the rat—particularly *Rattus norvegicus*—is a very frequent carrier of pathogenic leptospiræ.

In Japan 40·2 per cent. of 149 rats of this species were found infected (Ido *et al.* 1917*a*); in England 30 per cent. of 100 wild rats (Balfour 1922, Stevenson 1922); in Scotland 36·7 per cent. of 166 wild rats (Buchanan 1927) and 27·3 per cent. of 117 wild rats (Smith 1938); in Berlin 10 per cent. of rats (Uhlenhuth and Zuelzer 1921); in Toronto 37 per cent. (Cameron and Irwin 1929); in Freiburg 44 per cent. (Zimmermann 1930); in Moscow 12 per cent. (Ssinjuschina 1929); and in Kiew 31 per cent. (Basilewsky 1933). In Rotterdam, where cases of Weil's disease are fairly common, from 7 to 40 per cent. of all rats examined have proved to be infected (Schüffner 1934). The frequency of infection depends largely on the age of the rat. Young rats are seldom infected, while over 50 per cent. of adult rats may harbour the spirochætes. The black rat rarely acts as a carrier. Field-mice are not uncommonly infected (Ido *et al.* 1917*a*, Buchanan 1927).

The spirochætes are found in the kidney and the urine, not in the blood or liver. Intraperitoneal injection of rat's urine, even in small amounts, 0·1–0·2 ml., into guinea-pigs may give rise to fatal hæmorrhagic jaundice. Not all specimens of rat's urine in which leptospiræ are visible microscopically prove infective ; this is probably dependent on the acidity of the rat's urine, which rapidly proves fatal to the organisms. The same applies to the effect of human urine ; only in one-third of cases of Weil's disease does the urine prove to be infective. Uhlenhuth and Zuelzer (1921) found that fresh human or rat's urine rapidly killed the spirochætes ; but if the urine was neutralized, the organisms remained virulent for 2 days.

Leptospiræ in Water.—In view of the frequency of infection of rats' urine, it should be possible to demonstrate the presence of *Lepto. icterohæmorrhagiæ* in contaminated water.

Spirochætes morphologically identical with *Leptospira icterohæmorrhagiæ* are widespread in water. They were first observed by Wolbach and Binger in 1914, and have since been found by numerous workers in different parts of the world. In this country and in America they are known as *Leptospira biflexa* and in Germany as *Spirochæta pseudoicterogenes* (see Chapter 38). As pointed out by Zuelzer (1928) they are generally found attached to other spirochætes and protozoa. They are especially prevalent in the slime of ponds, lakes, and rivers, in the slime that collects on the ends of water taps and pipes, and in the roof slime of mines. They are very susceptible to acid and are therefore confined to waters with a pH of over 6·8. Thus they are abundant along the east coast of Sumatra where the water is alkaline and practically absent from Java where the water is acid (Sardjito and Zuelzer 1929). They are also susceptible to salt, and are said to perish in 3 days in alkaline water containing 0·17 per cent., and in a few hours in water containing 1·7 per cent., of chlorine as chloride (Schüffner 1934).

These organisms have occasioned much discussion. For some time opinion was influenced by the experiments of Baermann and Zuelzer (1927, 1928). These workers brought evidence to suggest that, though on first isolation water leptospiræ were avirulent, by passage through guinea-pigs, or occasionally through human beings, they could be so raised in virulence as to give rise in the guinea-pig to typical hæmorrhagic jaundice. In fact Baermann and Zuelzer regarded *Lepto. biflexa* merely as an avirulent form of *Lepto. icterohæmorrhagiæ*. Extensive observations by various workers have failed to confirm these findings. Possibly Baermann and Zuelzer were working with a strain of *Lepto. icterohæmorrhagiæ* that had become avirulent by residence under saprophytic conditions, or they may have isolated from one of their guinea-pigs a naturally infective strain entirely different from their passage strain. Except in special conditions, where the locality is heavily rat-infested, it is uncommon to isolate a pathogenic leptospiral strain from water. Since *Lepto. biflexa* is widely distributed over the globe, while cases of Weil's disease are topographically and occupationally circumscribed, it is difficult to avoid the conclusion that *Lepto. biflexa* has no ætiological relationship to the disease. Most of the evidence suggests that water becomes contaminated with *Lepto. icterohæmorrhagiæ* from rats' urine, and that even in favourable circumstances the organisms die out fairly rapidly. For this reason infection of human beings is unlikely except from water exposed to frequent and heavy contamination.

Summarizing, it may be said that the evidence points strongly in favour of infection occurring through contact, often close and prolonged, with water or sewage recently contaminated with the urine of rats. A few cases of laboratory infection by contamination of the conjunctiva or the abraded skin with infective guinea-pig material have been recorded (Uhlenhuth and Fromme 1916, Uhlenhuth and Zimmermann 1933).

Diagnosis.—According to Schüffner (1934), 60 per cent. of patients with Weil's disease show no jaundice. The true nature of the illness in these cases is suggested by the severe muscular pains at the onset, the heavily coated tongue, the albuminuria, the meningeal symptoms, the increase in the proportion of immature polymorphonuclear leucocytes, and probably most striking of all the flushed conjunctivæ due to dilatation of the episcleral capillaries. Cases without jaundice are not fatal.

The diagnosis has to be made finally by the bacteriologist. Several methods are available. In general the aim is to demonstrate the organisms in the blood during the 1st week of the disease and in the urine during the subsequent weeks, and to confirm the diagnosis by serological examination. Though leptospiræ can almost invariably be found by dark-ground illumination in the freshly drawn blood of infected guinea-pigs, they are rarely found by this method in human cases. Cultivation of the blood is much more successful, and in the hands of certain workers, such as Taylor and Goyle (1931), has yielded a high proportion of positive results; over 80 per cent. of positive cultures were obtained when the blood was withdrawn on the 4th or 5th day of the disease. Fletcher's medium (see Chapter 38) is recommended. Alternatively, 2–3 ml. of the blood may be inoculated intracardially or intraperitoneally into young guinea-pigs (see p. 922); the success of this method seems to depend on the virulence of the infecting strain. After the 1st week or 10 days of the disease, the organisms usually disappear from the blood and must be sought for in the urine. This should be examined by dark-ground illumination and by guinea-pig inoculation. The microscopical diagnosis is not always easy or reliable, since leptospiræ in acid urine are often atypical in shape and may be granular and degenerated (Buchanan 1927); for this reason it is advisable to give the patient potassium citrate to render the urine alkaline.

For ease and rapidity of diagnosis more and more attention is being paid to serological methods of examination. Agglutinins, lysins, and complement-fixing bodies appear about the end of the 1st week of the disease and increase to reach their maximum after 4–7 weeks (Kisker 1935, Wolff 1936).

Agglutination may be carried out on slides incubated at 32° C. for 3 hours and observed under dark-ground illumination, using a low-power objective and a compensating ocular. If living organisms are used, agglutination is seen to occur in the lower dilutions and lysis in the higher. With formolized and phenolized suspensions agglutination alone is seen. Two or three different strains should be used, including one of *Lepto. canicola*. In most laboratories now the microscopic slide method has given way to the macroscopic tube method of agglutination. At first, living suspensions were used (Smith and Tulloch 1937), but these were replaced by suspensions killed with formalin. A simple and reliable technique has been described by Gardner (1943), who grows the organisms for 3 to 4 days at 30° C. in a 12 per cent. rabbit serum water medium, adds 0·25 per cent. formalin, filters through cotton-wool, makes up dilutions of serum from 1/25 to at least 1/1,000, and incubates the mixture for 24 hours at 37° C

By the end of the 1st week of the disease the titre should be at least 1/20; during the 2nd week it rises, and often passes the 1/1,000 level; sometimes, at a later stage of the disease, it may reach even 1/50,000. Reactions at 1/25 or even 1/100 cannot be taken as diagnostic, though complete flocculation at the latter level is very suspicious. Patients showing less than 1/100 at a first test should be re-examined after five days or so to establish a rise of titre. The interpretation of the result must depend not only on the titre, but on the clinical findings,

the occupation of the patient, and the frequency of latent infections in the locality. Agglutinins persist for a long time, and a retrospective diagnosis may be made even years after the attack (see Schüffner 1930, 1932, 1934, Gaehtgens 1933, Bijl and Korthof 1930, Davidson *et al.* 1934, Packchanian and Tom 1943). The *adhesion phenomenon* (see p. 912) may be used instead of direct agglutination (Brown 1935). Agglutinins are often present in the urine in titres up to 1/250 (van der Hoeden 1935). The complement-fixation test has been reported upon favourably by Pot and Dornickx (1936).

Spirochætocidal antibodies may be tested for by mixing 1–2 ml. of the patient's serum with several lethal doses of the organism, allowing the mixture to stand at room temperature for 30 minutes, and then injecting it into guinea-pigs. If protective bodies are present the animals will survive, while control animals will die of spirochætal infection.

The occurrence of a polymorphonuclear leucocytosis in Weil's disease is said to be of value in the differential diagnosis from epidemic jaundice, in which a lymphocytosis is usually observed (see Murgatroyd 1943).

The demonstration of infection in *rats* may be accomplished by dark-ground examination and cultivation of the kidney and urine, by microscopical examination of sections of kidney stained with Levaditi, by inoculation of mice or guinea-pigs with ground-up kidney tissue; or indirectly by examination of the blood serum, or less successfully the urine (see van der Hoeden 1936), for antibodies. Agglutinins and lysins are often present in the serum in a titre of 1/400–1/3,000 (see Cameron and Irwin 1929, Zimmermann 1930). Larson (1943) obtained the highest proportion of positive results by examination of the blood serum, taking a titre of 1/100 or over as diagnostic; examination of stained sections of kidney came next, followed by dark-ground examination of the kidney. In examining rats for leptospiral infection, it is important that the animals should be freshly killed, since the spirochætes disappear rapidly after death (Smith 1938). For demonstrating the presence of *Lepto. icterohæmorrhagiæ* in *water*, the method described by Appelman and van Thiel (1935) of scarifying the shaved abdominal skin of guinea-pigs, and immersing them in the water for an hour, seems to hold out most prospect of success.

Prophylaxis and Treatment.

The prevention of Weil's disease would seem to consist in the drainage of infected areas and the suppression or exclusion of rats.

Prophylactic vaccination of workers who are specially exposed to risk of infection has been tried on a considerable scale in Japan with very promising results. The figures of Wani (1933), in particular, are impressive. He used a phenolized vaccine and gave two doses. The morbidity rate in the vaccinated subjects was only about a quarter of that in the control population. Little experience has been gained of vaccination in Europe. The few observations made by Smith (1937) in Aberdeen were disappointing, but more favourable results have been reported from Amsterdam (see Walch-Sorgdrager 1939).

Treatment by salvarsan is unsatisfactory; Weil's disease is in fact the only spirochætal infection that does not yield to arsenic. The most promising line of treatment is by immune horse serum; according to Martin and Pettit (1919), 60 ml. of serum should be injected subcutaneously on the 1st day, and 20–40 ml. on each of the next 2 or 3 days. In severe cases it should be given intravenously. According to Walch-Sorgdrager (1939), who reviews the effect of serotherapy,

there is insufficient evidence as yet to prove conclusively that the serum exerts a specific action upon the infecting organism, but there is little doubt that it has a beneficial effect when administered within four days of the onset of the disease. There is some evidence that the case mortality may be lowered (see Inada 1923). Experimental observations on the guinea-pig suggest that penicillin may be of value in treatment (Heilman and Herrell 1944*a*). (For much useful information on Weil's disease, the reader is referred to the monograph by Taylor and Goyle 1931, to papers by Alston and Brown 1937 and Walch-Sorgdrager 1939, and to the review by Ashe, Pratt-Thomas and Kumpe 1941).

Other Leptospiral Infections of Human Beings

Throughout the world there are a number of diseases simulating to a greater or lesser extent Weil's disease, but caused by leptospiræ that differ from *Lepto. icterohæmorrhagiæ* in their antigenic or pathogenic properties, and sometimes in their natural animal reservoir (see Chapter 38). No satisfactory classification of these diseases is yet possible and, as has already been pointed out, their nomenclature is in a state of confusion. We propose to group them tentatively according to our present inadequate knowledge of their clinical and epidemiological features, and to some extent according to the apparent affinities of their causative organisms (see Table 181). (For references to these diseases, see Walch-Sorgdrager 1939.)

Group A. Hasamiyami, and some diseases of the Dutch East Indies and the Andaman Islands resembling Weil's disease.

Hasamiyami is a disease found mainly among the peasantry of Nagasaki (see Kotorii 1935, Kaneko, Kotorii and Aoki 1935). Jaundice is fairly common, glandular swelling occasional, and clouding of the vitreous humour almost constant. The mortality is not more than about 2 per cent. The causative agent is *Lepto. autumnalis*, which appears to be identical with *Lepto. akiyami A.* The natural reservoir of infection is a field-mouse, *Apodemus speciosus.*

Cases of leptospiral infection simulating Weil's disease have been described among plantation workers in Sumatra caused by *Lepto. rachmat*, and among convicts and soldiers in the Andaman Islands, due to *Lepto. andaman A.* The animal reservoir of these two organisms is unknown, but it may well prove to be field-rats or mice.

An endemic infection among plantation workers in Sumatra due to *Lepto. salinem*, and sporadic infections among men and women in Batavia, Borneo, Macassar, and other parts of the Dutch East Indies, due to *Lepto. bataviæ*—sometimes known as the Swart van Tienen type—may be included in this group. *Lepto. salinem* is carried by *Rattus brevicaudatus*, and *Lepto. bataviæ* by *Rattus norvegicus.*

These diseases all resemble Weil's disease in some respects; all may give rise to jaundice and hæmorrhages; all may prove fatal, though as a rule the case mortality is low; and all are caused by organisms that are moderately virulent for the guinea-pig. There is some reason to believe that the agglutinins, instead of being demonstrable in the blood serum of patients for years after the attack—as in Weil's disease—may disappear completely within a year (Wolff 1936).

Group B. Nanukayami, Akiyami, Coastal fever of Queensland.

Nanukayami, sometimes known as *sakushu* fever or the *7-day fever of Japan*, is an autumnal disease occurring in many rural districts of Japan. Jaundice is slight or absent, and the disease is never fatal. We owe our knowledge of its

TABLE 181

SUMMARY OF THE KNOWN FORMS OF HUMAN LEPTOSPIRAL INFECTIONS (Modified from Walch-Sorgdrager, 1939).

Disease.	Clinical Symptoms.	Epidemiological Features.	Causative Organism.	Animal Inoculation.	Carrier.
Weil's	Jaundice frequent. Mortality variable, but often high.	Affects persons brought into contact with water contaminated by rats' urine, endemic in sewer and mine workers. Males much more often affected than females.	*L. icterohæmorrhagiæ.*	Virulent for guinea-pigs, producing jaundice.	Rats, *Rattus norvegicus* and *R. alexandrinus.*
Hasamiyami	Jaundice fairly frequent. Seldom fatal.	Endemic among peasants in Nagasaki during autumn. Men more often than women.	*L. autumnalis* (apparently identical with *L. akiyami A*).	Moderately virulent for guinea-pigs, often producing jaundice.	Field-mice, *Apodemus speciosus.*
Simulating Weil's disease	Jaundice fairly frequent. Mortality low. Vitreous opacities common	Endemic in Dutch East Indies. Mainly among plantation workers.	*L rachmat.*	Virulent for guinea-pigs, usually producing jaundice.	?
Simulating Weil's disease	Jaundice fairly frequent. Mortality low to moderate.	Among soldiers and convicts working in mud in the Andaman Islands. Chiefly in autumn.	*L. andaman A.*	Moderately virulent for guinea-pigs	?
Simulating Weil's disease	Jaundice not infrequent. Mortality low to moderate.	Endemic in Dutch East Indies, often in workers on plantations. Men and women affected.	*L satanem* and *L. bataviæ.*	Moderately virulent for guinea-pigs, often producing jaundice.	Rats, *R. brevicaudatus; R. norvegicus.*
Nanukayami or 7-day fever; Akiyami or Autumn fever of Japan.	Jaundice rare. Swelling of lymph glands. Not fatal.	Sometimes in epidemic form in rural areas of Japan during autumn.	*L. hebdomadis* (apparently identical with *L. akiyami B*).	Virulence for guinea-pigs slight or absent.	Voles, *Microtus montebelloi.*
Cane or Coastal fever of Queensland.	Jaundice rare. Glands often enlarged. Severe, but seldom fatal.	Cane-field workers in Queensland. Mainly men.	*L. australis A* and *B.*	Moderately virulent for guinea-pigs.	Cane-field rats, *R. culmorum.*
Weil-like disease . .	Jaundice uncommon.	Rural workers in Denmark.	*L. sejroe.*	Virulence for guinea-pigs very low.	Field-mice, *Mus spretegus.*
Swamp fever . . . Mud fever	Jaundice rare. Not fatal.	Epidemic and endemic among people working on swampy ground; common in late summer in Russia, Germany, Italy, Andaman Islands, etc.	*L. grippo-typhosa* (apparently identical with *L andaman B*).	Virulence for guinea-pigs very low or absent.	? Voles, *Microtus arvalis*
Pomona fever, or 7-day fever of Queensland.	Jaundice rare. Not fatal.	Among dairy workers in Queensland during summer and early autumn.	*L. pomona*	Virulence for guinea-pigs very low.	Endemic infection of pigs and cattle.
7-day fever of Queensland.	Jaundice rare. Not fatal.	Among workers with farm animals.	*L. mitis*	Virulence for guinea-pigs moderate	Endemic infection of pigs and cattle.
Weil's	Jaundice rare. Not fatal. May be meningeal symptoms	Infection from dogs.	*L. canicola.*	Virulence for guinea-pigs very low.	Dogs

bacteriology to the Japanese workers, Ido, Ito and Wani (1918, 1919). Th causative organism is *Lepto. hebdomadis*, and the natural reservoir of infectio the vole, *Microtus montebelloi*.

Akiyami, or the Autumn fever of Japan, is a disease similar to nanukayami Indeed one type of leptospira isolated from some of the cases, known as the *Lepto akiyami B* type, appears to be identical with *Lepto. hebdomadis* (Stéfanopoul and Hosoya 1928). The *A* type is apparently identical with *Lepto. autumnalis*—already mentioned as the cause of hasamiyami.

In northern Queensland there is a group of coastal fevers that are gradually being differentiated. From one group, common among workers in the sugar-cane fields, two types of leptospira have been isolated—*Lepto. australis A* and *B* (Lumley 1937). The *A* species is clearly distinct; the *B* species is closely related to *Lepto. salinem* (Walch-Sorgdrager 1939). The animal reservoir appears to be a field-rat —*Rattus culmorum*.

Another leptospiral infection that may be included in this group is a mild disease recorded in Denmark by Petersen and Christensen (1939) due to an organism named *Lepto. sejroe*. Country workers are mainly affected, and the natural animal reservoir appears to be a field-mouse—*Mus spicilegus*.

The diseases included in this group tend to be milder than those classified under Group A. They occur mainly among rural workers. The usual clinical picture is a febrile disease lasting about a week, seldom accompanied by jaundice, and rarely proving fatal. The causative leptospiræ have, as a rule, only a low degree of virulence for the guinea-pig, and the natural animal reservoirs are field-mice.

GROUP C. **Swamp Fever.**

This disease, which is also called by such names as Mud fever, Slime fever, Harvest fever, Field fever, or the German Schlammfieber, is widespread in eastern Europe. It has been studied particularly in Germany (see Kathe 1928, 1941, Korthof 1932, Rimpau 1937, Wolter 1939), and in Russia (Tarassoff 1935). It has been reported in the Andaman Islands, the *B* type of leptospira there being apparently identical with the causative organism of swamp fever, *Lepto. grippotyphosa*. Whether the disease reported among the rice-field workers of northern Italy by Mino (1937, 1938, 1939, 1942) and Babudieri (1939) is the same disease as swamp fever is difficult to say, but so far as can be ascertained more than one type of leptospira seems to be responsible. Swamp fever may occur in epidemics of considerable magnitude. Field workers in swampy districts are attacked, particularly during the hay harvest. Young adult males constitute a considerable proportion of the cases, presumably because of their greater exposure to infection from contaminated water. Clinically the onset is sudden with rigors or chills, headache, pains in the back and limbs, often severe cramps in the calves, and sometimes nausea, vomiting, and diarrhœa. In a minority of the cases a macular eruption appears on the body and face on the 3rd or 4th day. Jaundice is uncommon. The disease lasts for 5–7 days, the temperature falling by lysis. There is sometimes a short relapse 1 or 2 days later. The case mortality is about 0·4 per cent. (Korthof 1932). The disease can be reproduced in human subjects by inoculation with pure cultures of the infecting organism. Leptospiræ can be demonstrated in the blood during the first 2 days of the disease. In practice bacteriological diagnosis is best made by examination of the blood serum for agglutinins, which often reach a titre of over 1/1000 by the 2nd week. The

virulence of *Lepto. grippo-typhosa* for guinea-pigs is very low or absent. The animal reservoir is not known with certainty, but the vole (*Microtus arvalis*) is suspected (Schüffner and Bohlander 1942).

The disease in man is not to be confused with the *swamp fever* or *infectious anæmia of horses*, which is apparently due to a filtrable virus (Stein 1935) (see p. 1966).

It will be realized that the diseases in this group are often characterized by gastro-intestinal symptoms; jaundice is uncommon, and death rarely occurs. The causative organism is almost non-virulent for guinea-pigs.

GROUP D. **Seven-day fever of Queensland, and other diseases.**

Clayton and Derrick (1937) described a 7-day fever in Queensland due to an organism named *Lepto. pomona*. Later Johnson (1942) described a very similar disease—also in Queensland—due to a different organism, *Lepto. mitis*. Both these diseases occur mainly in farmers and workers who are in contact with farm animals. The disease is mild, jaundice is rare, and death does not occur. Whether there is a rodent carrier is still unknown, but both types of leptospira have been found to be endemic in pigs and cattle.

Another disease that may be considered here is a febrile infection due to *Lepto. canicola*, an organism whose natural carrier is the dog. The disease caused by this organism has been met with sporadically in different parts of the world. It is usually mild, though sometimes chronic, is unaccompanied by jaundice, and is not fatal. Meningeal symptoms may occur.

The diseases classified in this group are similar in their mildness to those in Groups B and C. Infection, however, seems to be contracted not from rodents, but from the larger domestic animals. The causative organisms have, as a rule, only a very low degree of virulence for guinea-pigs.

SPIROCHÆTAL JAUNDICE IN ANIMALS

We have already mentioned that about 20–30 per cent. or so of wild rats are infected with *Lepto. icterohæmorrhagiæ*. The organism apparently gives rise, however, to little systemic disturbance in these animals, and rarely causes jaundice.

A disease, similar to Weil's disease and accompanied by jaundice, is not infrequent in **dogs** (see Okell *et al.* 1925). It appears to be caused, as a rule, by *Lepto. icterohæmorrhagiæ*. More frequently, however, leptospiral infection in dogs is due to *Lepto. canicola*, and takes the form, not of jaundice, but of uræmia (Stuttgart dog plague, canine typhus of Lukes). The disease caused by *Lepto. icterohæmorrhagiæ* affects dogs up to 3 years of age, runs an acute course, is accompanied by jaundice, and has a case mortality of about 50 per cent. The disease caused by *Lepto. canicola* tends to attack slightly older dogs, runs a more chronic course, is characterized by an azotæmic-uræmic syndrome due to affection of the kidneys, is seldom accompanied by jaundice, and has a case mortality varying from 40 to 80 per cent. (see Dhont *et al.* 1934, Schüffner *et al.* 1935, Klarenbeek 1938, Niemand 1940). There is, however, no sharp line of demarcation between the two diseases. Infections with *Lepto. canicola* appear to be twice as frequent as those due to *Lepto. icterohæmorrhagiæ* (Klarenbeek 1938). The disease due to *Lepto. canicola* is much commoner in dogs than in bitches, partly because this organism is excreted in the urine more often than *Lepto. icterohæmorrhagiæ*, and partly because of the habit so common in dogs of sniffing the urine or external

genitalia of other dogs (Walch-Sorgdrager and Schüffner 1938). Dogs may act as healthy carriers of leptospiræ; Kouwenaar and Wolff (1930) in Sumatra found 5·7 per cent. of street dogs to be infected. Such dogs constitute a source of danger for man. Bacteriological diagnosis follows the general lines already laid down for the human disease; demonstration of agglutinins and lysins in the dog's serum is a rapid and convenient method (van der Hoeden 1935).

Leptospiral infections have been encountered in foxes (Dunkin and Laidlaw 1924–25), in mice (Bessemans and Thiry 1929), in cats (Esseveld and Collier 1938), and in certain other animals.

RAT-BITE FEVER

As has already been pointed out (p. 1276), there appear to be two distinct diseases known as rat-bite fever, one due to *Spirillum minus*, the other to an organism generally referred to as *Streptothrix moniliformis*, but better called *Actinomyces muris*. We shall consider here only the spirillar type of infection.

Rat-bite fever, or *Sodoku* as it is called in Japan, is a disease that occasionally supervenes in man on the bite of a rat, or occasionally some other animal such as a cat (Mollaret and Bonnefoi 1938, Yamamoto 1939). The incubation period is generally 7 to 21 days, but it may extend to weeks or even months. The disease is ushered in by a sharp febrile paroxysm accompanied by swelling of the lymph glands and dark-red eruptions on the skin. Redness and swelling are noticed at the site of the wound, which during the incubation period has generally healed satisfactorily. There are often pains in the limbs on the affected side. After 3 or 4 days the attack comes to an end, but is succeeded by another in a few days. These febrile paroxysms with intermittent afebrile periods may be repeated for months, or even years. The mortality of the disease varies from about 2 to 10 per cent.

The causative organism of the disease is a *Spirillum*, which was described in 1916 by Futaki and his co-workers (Futaki *et al.* 1916) in Japan (see p. 1838). The organism has been found in cases of rat-bite fever in this country (Robertson 1924), in Italy, Turkey, India, Dutch East Indies, and the United States (Shattock and Theiler 1924, Brown and Nunemaker 1942, Beeson 1943). (For a review of the literature see Robertson 1924, Theiler 1926, McDermott 1927–28, Brown and Nunemaker 1942.) The disease has been reproduced experimentally in human beings by inoculation with infected guinea-pig blood (see Hershfield *et al.* 1929). The clinical differentiation of the disease caused by *Sp. minus* from that caused by *Actino. muris* has been studied by Allbritten, Sheely and Jeffers (1940) and Brown and Nunemaker (1942), and is referred to on p. 1278. In tsutsugamushi fever (see p. 1849), with which rat-bite fever may be confused, the local ulcer is said to be larger, more irregular in outline, and painful, and the elements of the rash on the trunk are of greater size (Lewthwaite 1940).

In the human patient the organism is present in the swollen local lesion, the focal lymph glands, and the blood, but it is very difficult to demonstrate except by animal inoculation; at autopsy it can be found also in the kidneys (Kaneko and Okuda 1917). Microscopical examination of the blood is usually negative. The Wassermann reaction is said to be positive in about half the cases (Brown and Nunemaker 1942); and agglutinins to *Proteus OX* K may be present (Lewthwaite 1940). The bacteriological diagnosis is best made by subcutaneous or

intraperitoneal inoculation of blood taken at the height of a febrile paroxysm, or of serum expressed from the local lesion, into mice and guinea-pigs (Francis 1932). Aspirated peritoneal fluid or blood should be examined daily from the 5th to the 30th day by dark-ground illumination. Brown and Nunemaker (1942) recommend the inoculation of at least 2 guinea-pigs and 4 mice. The guinea-pigs should be inoculated intracutaneously, subcutaneously, and intraperitoneally. Intracutaneous inoculation is sometimes followed by a chancre, and subcutaneous inoculation by enlargement of the regional lymph nodes; in both of these sites spirilla may be demonstrated by dark-ground examination. It is important to remember that mice and less often guinea-pigs may be healthy carriers of *Sp. minus*, so that it is wise to take precautions against confusion resulting from this cause.

Numerous strains of *Spirillum minus* have been isolated from mice, rats, and human patients; according to Schockaert (1928) they are all similar, constituting a single species. In Japan about 3 per cent. of house rats appear to carry the organism (Futaki *et al.* 1917). According to Manuouélian (1940) the spirilla are present in considerable numbers in the muscles of the tongue, which may account for the infectivity of the bite. In the serum of patients who have recovered from rat-bite fever spirochæticidal bodies have been found (Ido *et al.* 1917*b*). Salvarsan or neoarsphenamine usually cuts the fever short, but complete cure of the disease is not always easy to obtain. Recent studies, however, suggest that it may respond to penicillin treatment (Lourie and Collier 1943, Heilman and Herrell 1944*b*).

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CHAPTER 83

TYPHUS FEVER AND OTHER RICKETTSIAL DISEASES

THERE must be few diseases about which our knowledge has increased so rapidly during the past two decades as the typhus fever group. Though epidemic typhus has been known for centuries, and has played no small part in determining the course of history it was not till 1909 that Nicolle and his colleagues (1911) first discovered that infection was spread by the body louse, and not till 1916 that da Rocha-Lima demonstrated the ætiological agent of the disease, *Rickettsia prowazeki*. In 1907 Ricketts in the United States had succeeded in transmitting Rocky Mountain spotted fever to guinea-pigs, but the generic relationship of this disease to typhus fever remained obscure till Wolbach (1925) in 1916 demonstrated the rickettsial nature of the causative agent. During the war of 1914–18 a new fever, referred to as Trench fever, was met with; this was likewise found to be due to a species of *Rickettsia*. A sporadic form of typhus fever known as Brill's disease had been recognized for a long time in New York, a severer form known as *Tabardillo* in Mexico, and a milder form in Manchuria and the Far East. Broadly speaking, however, up till 1925, typhus fever was regarded as one of the major epidemic diseases, which in certain areas might sometimes occur in a relatively mild sporadic form. Rocky Mountain spotted fever and Trench fever were regarded as rickettsial diseases due to species of *Rickettsia* entirely different from that causing typhus fever.

In 1925 Fletcher and Lesslar (1925, 1926) found that the endemic typhus fever of the Federated Malay States could be divided serologically and epidemiologically into two entirely different groups, one met with in the towns, the other occurring in country districts. Both differed in certain important respects from the classical type and from each other. These observations provided the main impetus for an extensive and detailed search in various parts of the world for other types of typhus fever. The result has been to reveal the existence in several different countries of endemic typhus fever or typhus-like fevers many of which had previously been unrecognized.

We shall anticipate our discussion on the classification of the various rickettsial diseases of man by describing the diseases themselves, grouping them into four main categories: the typhus fever, spotted fever, tsutsugamushi, and Q fever groups, to which will be added trench fever.

THE TYPHUS FEVER GROUP

Two main varieties of typhus fever are known. The first is often referred to as the classical type; it is caused by *R. prowazeki*, and appears to be invariably louse-borne. The second is called the murine type; it is caused by *R. mooseri*,

and is usually carried to man by the rat flea. Each of these may be briefly considered in turn.

Classical Typhus Fever. *Synonyms:* Fleckfieber, Exanthematische Typhus (German).

This type, though now uncommon in epidemic form in civilized countries, has been in the past one of the great scourges of the world. Like dysentery, it appeared wherever large numbers of people were herded together under insanitary conditions; for this reason it was common in gaols—hence its alternative name " gaol fever "—in military campaigns, and during times of famine. It was specially prevalent in Russia and in Central Europe during the war of 1914–18; and even in the second world war, when protective measures were much better developed, it was a constant cause of trouble and anxiety. The case mortality varies with age. In severe epidemics it is negligible in children, seldom more than 5 per cent. below 20 years of age, about 10–15 per cent. at 40 years, 50 per cent. at 50 years, and generally fatal over 60 years of age (Megaw 1942). Those who wish to realize something of the havoc the disease has wrought among the human population, and of the significant part it has played in determining the outcome of great military campaigns, will do well to refer to the fascinating account given by Zinsser (1935).

In 1909 Nicolle and his colleagues (1911), by experiments on man and on monkeys, showed that the disease was spread by the body louse, *Pediculus corporis*. The louse becomes infected by ingesting the blood of the patient. Within a period varying from about 1 to 2 weeks after feeding, the louse becomes infective, and can transmit the disease to normal persons. The time during which lice may remain infective is not known with certainty, but da Rocha-Lima (1920*a*) has demonstrated their infectivity for at least 24 days, and he believes that they remain infective for the rest of their lives. The great majority, however, of infected lice die within a fortnight It is believed that head lice as well as body lice, but not pubic lice, may act as natural vectors.

The maintenance of infection during non-epidemic times has given rise to much speculation. It is possible that a few mild or symptomless sporadic cases may occur, just sufficient to keep a small number of lice infected (see Kuteischikow *et al.* 1933, Isabolinski *et al.* 1934, Mooser 1941*b*). Alternatively, it is supposed that some persons remain latently infected for considerable lengths of time (see Afanassiewa and Tretjak 1933), and develop a mild disease, perhaps some months after their original infection. The evidence in favour of this second explanation has been carefully marshalled by Zinsser (1934) in his study of *Brill's disease*. This is a very mild form of endemic typhus, which occurs in the north-eastern coastal towns of the United States, and is restricted almost entirely to immigrant Russian and Polish Jews. Zinsser found that infection was caused by the classical type of typhus virus, which must almost certainly have been acquired before the patients had left Europe. The disease represents, in fact, the recrudescence of a latent infection acquired at some earlier time. A third possibility is that infection persists in dried louse fæces attached to patients' clothing and is transmitted to fresh subjects by inhalation. There is reason to believe that the virus may remain alive for a year or more under these conditions, and the high infectivity of airborne rickettsiæ has been amply demonstrated by laboratory observations (see Löffler and Mooser 1942, van den Ende *et al.* 1943).

Bacteriology.

The causative agent of typhus fever is *Rickettsia prowazeki*, first described by da Rocha-Lima in 1916, and later in the same year by Töpfer (1916*b*). Da Rocha-Lima observed this organism in the lice taken from 95 per cent. of typhus patients; it was present in enormous numbers in the intestinal lumen and in the epithelial cells lining the gut wall (Fig. 297). The organisms stain a reddish-purple with Giemsa, are very small, of short elliptic or olive-shape, often in pairs, and are surrounded by a paler staining substance; occasional very short, and very long forms up to 1·5–2 μ, may be seen. They were never found in lice fed on normal persons, or on typhus convalescents, but appeared regularly, and in enormous numbers, in lice fed on typhus patients. The time elapsing between feeding and the appearance of rickettsiæ in the gut depended on the temperature at which the lice were kept; at 23° C. no rickettsiæ appeared at all, but at 32° C. they appeared in about 5 to 8 days, and persisted indefinitely. At about the same time as the rickettsiæ developed, the lice became infective for guinea-pigs. It is important to note that not every louse fed on a typhus patient develops rickettsiæ, or becomes infective. Wolbach, Todd and Palfrey (1922), for example, working in Poland in 1920, fed batches of 30 to 40 lice on typhus patients for an average period of 12 to 16 days, and found that, out of 52 experiments, in only 27 did the lice develop rickettsiæ. In a later series of experiments they also found that only a similar proportion of lice became infective for guinea-pigs. The actual proportion observed was very low, and is probably accounted for by imperfect technique in dealing with the lice; during the latter part of their work the proportion of positive results was considerably higher. Arkwright, Bacot and Duncan (1919) similarly concluded that rickettsiæ do not appear regularly in the excreta of lice fed on typhus patients. Whether this is due to variation in the lice, or, as seems more probable, to the relatively low infectivity of typhus blood, is not known. The significant point is the general parallelism that exists between the presence of rickettsiæ in the louse, and the infectivity of the louse for guinea-pigs.

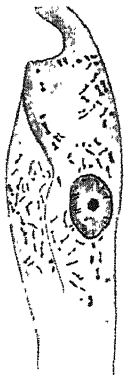


FIG. 297.—*Rickettsia prowazeki*, contained in an epithelial cell from the louse's mid-gut; both coccal and bacillary forms can be seen. Magnification 1,200 diameters (approx.). (After Wolbach, Todd and Palfrey.)

R. prowazeki has been cultivated by Wolbach and Schlesinger (1923–24) in tissue cultures. Small pieces of the brain of typhus guinea-pigs were seeded into normal guinea-pig plasma, and incubated in the usual way over hollow-ground slides. Rickettsiæ were found in the endothelial cells of the vessel walls; primary cultures remained virulent for 8 to 15 days, second generation cultures up to 28 days. The rickettsiæ showed signs of multiplication within the cells; they appeared as coccoid, bacillary, and filamentous forms similar to those found in lice.

Arkwright and Bacot (1923), using Weigl's technique, were able to infect lice by intra-rectal inoculation of a suspension of blood platelets from typhus patients and from infected guinea-pigs. To obtain regular results a thick suspension was

required—platelets from 2 ml. of blood suspended in 6 drops of saline. Similarly successful results were obtained by intra-rectal inoculation of lice with the excreta of infected lice.

Pathologically, both in man and in experimentally infected animals, the brunt of the attack is borne by the blood capillaries, especially those of the skin, skeletal muscles, and central nervous system. The primary site of invasion is the endothelial cells lining the walls of the capillaries. As a result, multiple thromboses occur with perivascular accumulations of cells, often accompanied by small hæmorrhages. In the central nervous system characteristic nodules are found, simulating tubercles. The demonstration of rickettsiæ in human patients is not easy, but they have been found in the lesions of the skin, kidneys, testicles, brain, and other organs in man (Wolbach *et al.* 1922, Wolbach 1925, Cowdry 1926). It will be realized that many of the signs and symptoms of typhus fever, such as the rash and nervous manifestations, are explicable in terms of the vascular lesions.

Relation of Rickettsia prowazeki to Typhus.—It may be well to set out the evidence on which the ætiological relationship of *Rickettsia prowazeki* to typhus is based.

(1) The occurrence of *R. prowazeki* in the characteristic perivascular nodules of typhus; (2) its occurrence as the sole distinctive acquisition of lice fed on typhus; (3) the failure to separate *R. prowazeki* from the virus of typhus by any method yet employed; (4) the cultivation of *R. prowazeki* in lice by the intra-rectal inoculation of a platelet suspension from typhus patients or infected guinea-pigs, even after the virus has been passed several times through guinea-pigs (Atkin and Bacot 1922); and (5) the cultivation of *R. prowazeki* in tissue plasma cultures, using as a source of the virus a strain that had been maintained for 3 years by passage through guinea-pigs: all suggest that *R. prowazeki* is the ætiological agent of typhus (Wolbach 1925). To these we may add that both the typhus virus and *R. prowazeki* are destroyed by exposure to moist heat at 50°–55° C., and that neither is capable of passing through any but the coarsest bacterial filters, and then only irregularly. Against this view it may be said that rickettsiæ are sometimes found in lice taken from persons not suffering from typhus. For example, da Rocha-Lima (1920*a*) found rickettsiæ closely simulating *R. prowazeki* in lice that had been fed on trench-fever patients and on normal persons; Arkwright and Bacot (1923) in Egypt had much the same experience; in some of the lice taken from 2 out of 12 apparently healthy persons they found rickettsiæ simulating *R. prowazeki*. The lice in which they were found were not infective for guinea-pigs, and it is probable that the rickettsiæ were different from those associated with typhus. Da Rocha-Lima considers that the rickettsiæ found on persons not suffering from typhus belong to a different species—generally known as *R. rocha-limæ*. He distinguishes three types of *Rickettsia* in the louse: (1) very fine forms, predominantly intracellular, not very closely packed—*R. prowazeki*: (2) thicker, exclusively extracellular, but lying on the cells—*R. quintana*: and (3) thicker forms, grouped in compact masses, occurring both intra- and extra-cellularly—*R. rocha-limæ*; these may perhaps be pathogenic organisms peculiar to the louse.

It will be seen that the cumulative evidence points very strongly to the ætiological rôle of *R. prowazeki* in typhus. Though rigid adherence to Koch's postulates demands that this conclusion must remain unproven till pure cultures, free from living cells, can be obtained, and used to reproduce the experimental disease in susceptible animals, we shall not depart from justifiable inference if we accept provisionally its essential truth. The onus now rests rather on those who would disprove it than on those who accept it. For a review of typhus fever, see Clavero and Gallardo (1941).

Murine Typhus Fever.

The recognition of an endemic form of typhus fever in the United States, occurring sporadically in man, and spreading not by the louse but by the rat flea *Xenopsylla cheopis*, was due to the brilliant work of a group of American and Mexican workers, prominent among whom were Maxcy, Mooser and Dyer (see Maxcy 1926, 1928, 1929, Mooser 1928, Mooser *et al.* 1931, Dyer *et al.* 1931*a*, *b*, *c*, *d*, 1932*a*, *b*, *c*, Ceder *et al.* 1931, Rumreich 1933). The disease is endemic, occurs in the summer and autumn, is commonest in seaports along the Atlantic border, is not associated with overcrowding, has no predilection for the lower classes, is non-contagious, and has a case mortality of only 1 per cent. It is caused by a virus, often referred to as *R. mooseri*, which differs in some respects from *R. prowazeki*, and which is responsible for causing an extremely mild endemic infection of rats. Sporadic cases in man are liable to occur in persons brought into close contact with these animals, infection being transferred by the rat flea *X. cheopis*. The virus is passed from rat to rat by the rat louse, *Polyplax spinulosus*, and by rat fleas. The rats are never very sick, and the fleas may harbour the virus for over a month and recover—both circumstances indicating a well-established and possibly ancient parasitism (Zinsser 1937). On the other hand, there seems very little doubt that, under suitable conditions, the disease may become epidemic in man and be spread by the body louse. This is apparently what has happened in Mexico where, in addition to sporadic cases of flea-borne typhus, an epidemic disease known as *Tabardillo* occurs. Like classical typhus, it has its maximum prevalence in the winter, is associated with overcrowding, is most prevalent in the lower classes of the population, is contagious, is carried by lice, and has a fairly high case mortality.

The demonstration by American workers of the rôle played by the rat and the rat flea in the spread of endemic typhus was soon followed by the recognition of the disease in other parts of the world. It is called by various local names, such as Toulon typhus, endemic typhus of Moscow, Manchurian typhus, shop typhus of Malaya, and so on. Unlike the classical form, which is commonest in temperate climates, the endemic murine form of typhus tends to be more prevalent in warm countries. Examination of wild rats in numerous areas where the disease is endemic has shown that these animals often give a positive Weil-Felix reaction to *Proteus OX* 19, and that they are latently infected with a murine typhus virus (Penfold and Corkill 1928, Marcandier *et al.* 1931, Kodama and Takahashi 1931, Kodama *et al.* 1932*a*, *b*, Brumpt 1932*c*, Netter 1932, Lépine 1932, Lawton and Murray 1933, Chao-Jen Wu 1933, Kritschewski and Solowiow 1934, Suzuki 1934, Nicolle and Sparrow 1934, Epstein and Silvers 1934, Zinsser 1937, Wolbach 1941 ; see also Tonking 1932–33).

Comparison of the Classical and the Murine Typhus Viruses.—The two viruses of typhus fever, *R. prowazeki* and *R. mooseri*, are very closely related. Both stimulate the production of agglutinins acting on *Proteus OX* 19. Cross-immunity studies have often failed to elicit any difference between them (Zinsser and Castaneda 1930*a*, *b*, 1932, 1934, Zinsser and Batchelder 1930, Mooser and Dummer 1930, Weigl and Hertzig 1933, Mooser *et al.* 1934, Finlayson and Grobler 1940*a*, *b*) ; though there is reason to believe that, at any rate in man, a vaccine prepared with the murine type confers less protection against infection with the classical type than a vaccine prepared from the classical type itself. Their differentiation

is based mainly on the inability of the classical virus to give rise to a scrotal reaction in the guinea-pig or to a febrile disease in the rat (see pp. 934, 935). Pathologically, the two diseases are distinguished by the greater frequency of cerebral nodes in the classical type (see Lillie 1931, Pinkerton 1931). The difference in the case mortality between the two forms may be more a reflection of a difference between epidemic and endemic forms of disease than the result of intrinsic differences between the two viruses. It is known, for example, that in sporadic cases of typhus due to the classical virus, as in Brill's disease, the case mortality is often very low; and that in epidemic typhus due to the murine virus, as in Tabardillo, the case mortality may be high. How far the characters of the two viruses are fixed, it is impossible to say. Probably no one has succeeded in converting one into the other, but temporary modifications can be impressed on them by various means. Whether their behaviour can be so modified that they become indistinguishable is very doubtful, though Mooser (1941*a*) apparently regards this as a possibility.

It may be remarked that both types of virus are highly infective for laboratory workers. Inhalation of infective droplets or dust is very dangerous (see Löffler and Mooser 1942, van den Ende *et al.* 1943). The disease following inoculation of the murine type may vary from a severe to a completely inapparent infection (see Sparrow and Mareschal 1940, van den Ende *et al.* 1943); the disease following the classical type is usually more serious and has proved fatal to several laboratory workers.

THE SPOTTED FEVER GROUP

This group includes Rocky Mountain spotted fever, Brazilian spotted fever (São Paulo typhus), Fièvre boutonneuse, Kenya fever, and South African tick fever. Infection is carried not by lice or by rat fleas but by ticks. There is considerable variation in the severity of these diseases, but they are all characterized by fever and usually by a rash. In fièvre boutonneuse and South African tick fever, but not in the other three forms, there is a primary sore at the site of the tick bite, accompanied by inflammation of the regional lymph nodes. Other tick-borne rickettsial fevers, whose relationship to the spotted fever group is not very clear, have been described in India (see Megaw 1921, 1924, 1930, Boyd 1935, Covell 1936, Topping *et al.* 1943). In addition, there are a number of tick-borne diseases in North America—excluding tick-borne tularæmia and relapsing fever—the origin of which remains obscure, such as Tick paralysis, Colorado tick fever, and Bullis or Lone Star fever (see Parker 1938, Topping *et al.* 1940, Woodland *et al.* 1943, Humphreys *et al.* 1944, Florio, Stewart and Mugrage 1944).

Rocky Mountain Spotted Fever.

This disease, which has been known for many years, occurs in North America. Two types used to be recognized: a Western type with a case mortality of 70 per cent. or so; and an Eastern type occurring nearer the Atlantic border with a case mortality of only 20–25 per cent. (Badger 1933*a*). Recent workers, however, have cast doubt on this difference and tend to regard them both as one disease (Parker 1938, Wolbach 1941). Rocky Mountain spotted fever affects those engaged in outdoor pursuits. It has a striking seasonal incidence, being commonest during the summer and practically absent during the winter months (Hampton and Eubank 1938). The infecting agent, *Rickettsia rickettsi* (*Dermacentroxenus*

rickettsi), is carried by ticks. The Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, seems to be mainly concerned in the west and the American dog tick, *Dermacentor variabilis*, in the east (Dyer *et al.* 1931*e*). Some cases—usually mild—appear to result from the bite of the rabbit tick, *Hæmaphysalis leporis-palustris*, which is very widespread (see Parker 1938); and there is evidence that others may follow the bite of the Lone Star tick, *Amblyomma americanum*—so called from the star-shaped marking on its back—which occurs on dogs and cats, and which bites man in all stages of its development from the larva to the adult (Parker *et al.* 1943). The severity of the disease varies greatly. In mild cases the incubation period ranges from 3 to 14 days, in severe cases from 2 to 5 days. The fever lasts as a rule for 2 to 3 weeks. Convalescence is prolonged.

Ricketts (1907) was successful in transmitting infection to guinea-pigs and monkeys. Besides showing a febrile reaction, inoculated guinea-pigs often develop swellings and hæmorrhages of the scrotum and ears, which may go on to necrosis; the spleen is considerably enlarged (see Munter 1928). The Western type often proves highly fatal to guinea-pigs. Rabbits also develop a febrile disease and sometimes a scrotal reaction; introduction of the virus into the anterior chamber of the eye is less often followed by an acute reaction than occurs with the scrub typhus or tsutsugamushi virus (Lewthwaite and Savoor 1936*c*). Rats and mice can be infected, but the disease pursues a symptomless course (Fukuda 1929).

In human and animal lesions the organisms are found with comparative ease. The lesions themselves resemble those of typhus, but according to Lillie (1931, 1941), endovascular proliferation, degeneration, and thrombosis tend to be commoner. Moreover, in Rocky Mountain spotted fever the medial coat of the blood vessels is said to be affected as well as the endothelial lining (see Wolbach 1941). The blood vessels of the skin and reproductive organs suffer most severely.

The organism can be grown in tissue culture, and is distinguished from *R. prowazeki* by its multiplication in the nuclei of infected cells, where it forms compact spherical colonies (Pinkerton and Hass 1932). The typhus virus multiplies only in the cytoplasm. In the tick the organism presents three forms: (1) an intracellular bacillus-like form, without chromatoid granules, present only in the cytoplasm of the cells of the alimentary tract; (2) a relatively small, rod-shaped form with chromatoid granules, present within the cells and the nuclei of many tissues; (3) a relatively large lanceolate diploid form, which persists in the tissues, notably the salivary glands, long after the other forms have disappeared; this lanceolate form is characterized by its chromatoid staining reaction; it is the only form found in the eggs of infected ticks (see Wolbach 1925). It may be noted that ticks, once infected, are able to hand down infection to their progeny.

Brazilian Spotted Fever.—SYNONYM: São Paulo Typhus.

In its general symptomatology and high case mortality—70 per cent. or so—this disease resembles the severe type of Rocky Mountain spotted fever. Likewise, the Brazilian virus is similar to the Rocky Mountain virus in its peculiarly high pathogenicity for guinea-pigs and in the necrotic scrotal reaction to which it often gives rise in these animals and in rabbits. Inoculation into the anterior chamber of the rabbit's eye is not usually successful in causing an acute reaction; the guinea-pig's eye, however, is very much more susceptible, and an inflammatory reaction associated with corneal opacity develops 2–5 days after inoculation (Monteiro 1931, Dyer 1933). Cross-immunity tests leave little doubt that the São Paulo virus is identical with the virus of Rocky Mountain spotted fever (Dyer

1933, Parker and Davis 1933). Infection appears to follow the bite of the tick *Amblyomma cajennense* (Monteiro *et al.* 1932). (For general account of disease see Piza *et al.* 1932, Fialho 1932.)

Fièvre boutonneuse.

This disease, which was first described by Conor and Bruch (1910), occurs in Tunis and in certain other parts of the Mediterranean littoral. It appears to be identical with the *exanthematic fever of Marseille.* The name " boutonneuse " refers to the local nodule that is so often seen on the leg in the early stage of the disease. The clinical course is brief and mild, and the mortality is low. Infection is carried by the dog tick, *Rhipicephalus sanguineus.* Dogs serve as the reservoir of the virus. As many as 30 per cent. of these animals may be latently infected. Dog ticks may remain infective for several months, and can transmit the virus hereditarily to their progeny. Rickettsiæ have been found in infected ticks, and the name *Rickettsia conori* has been suggested for the causal agent (Brumpt 1932*b*) ; but since this organism is indistinguishable in tissue culture from the Rocky Mountain spotted fever virus (Hass and Pinkerton 1936), and since there appears to be complete cross-immunity between the two diseases (Badger 1933*b*, Blanc and Baltazard 1937), it is doubtful whether it is justifiable to accord it a specific name. Intraperitoneal inoculation of guinea-pigs often gives rise to a mild scrotal reaction (see Blanc and Caminopetros 1931, Durand 1931, 1932, 1933, Durand and Conseil 1931, Durand and Laigret 1932, Nicolle and Laigret 1932, Brumpt 1932*b*, Caminopetros and Contos 1932, 1933, Combiesco and Zotta 1932, Petzetakis and Karalis 1933, Combiesco 1934).

Kenya Fever and South African Tick Fever.

Neither of these diseases has been studied adequately. Kenya fever resembles fièvre boutonneuse, but differs from it in the absence of a primary sore. Infection appears to be carried by the dog tick, *Rhipicephalus sanguineus* (see Roberts and Tonking 1933).

South African tick fever is usually a mild disease, with a case mortality of only 1–2 per cent. It is characterized by a primary sore at the site of the tick bite and by regional adenitis. Infection is transmitted by the larvæ of three different ticks —*Amblyomma hebræum, Rhipicephalus appendiculatus,* and *Böophilus decoloratus*—and by all stages of the dog tick, *Hæmaphysalis leachi* (see Gear 1941). The virus gives rise to a mild fever in guinea-pigs, with little or no scrotal reaction. It can apparently be cultivated on the chorio-allantoic membrane of the developing chick embryo (Alexander, Mason and Neitz 1939). According to Pijper (1936) no cross-immunity exists between tick-bite fever and fièvre boutonneuse, and according to Pijper and Crocker (1938) there appears to be little relation between tick-bite fever and Rocky Mountain spotted fever. Both of these conclusions, however, are disputed by Mason and Alexander (1939*a*, *b*), who regard tick-bite fever as a member of the spotted fever group, and suggest that the virus should be given the varietal name of *pijperi.*

THE TSUTSUGAMUSHI GROUP

Three diseases have been described as belonging to this group : the tsutsugamushi fever of Japan, the scrub typhus of Malaya, and the mite typhus of Sumatra. There is evidence, however, that all three are essentially the same disease, caused by the same organism.

Tsutsugamushi, sometimes known as *Japanese river fever* or *Kedani*, occurs in Japan. It is commonest in the summer and autumn months; it is confined to certain localities, particularly to low-lying territory subject to flooding; and it follows the bite of *Trombicula akamushi* Brumpt—a mite found on the vole, *Microtus montebelloi*. This animal constitutes the reservoir of infection. The causative agent is *Rickettsia nipponica* (*R. orientalis, R. tsutsugamushi, R. akamushi*). It can be grown in tissue culture, but, unlike the spotted fever virus, it does not invade the nuclei. The disease is characterized by fever, a local swelling at the site of the puncture wound, enlargement of the lymph nodes and spleen, and a rash. The incubation period is about a week, and the case mortality is said to be 40 per cent. (Ogata 1931).

Scrub typhus is a disease met with in Malaya. It occurs at all times of the year; it attacks those who have been over country covered with a tall grass, known locally as lalang; infection follows the bite of *Trombicula deliensis*—a mite closely allied to, if not identical with, *Trombicula akamushi*—and rats appear to constitute a reservoir of infection (Lewthwaite and Savoor 1936*d*, 1940*b*). The incubation period is said to be 11–21 days, the duration of fever about a fortnight, and the case mortality 5–15 per cent. In the past it has been stated that this disease is distinguished from tsutsugamushi by the absence of an initial sore at the site of infection; but Lewthwaite and Savoor (1936*e*), who made special observations on this point, found that a sore was commonly present in Europeans, but was very apt to be overlooked on the dark-skinned Tamil. Moreover, a primary sore cannot uniformly be demonstrated in tsutsugamushi itself. It would appear as if the dermal lesion in the two diseases was subject to variation, being conspicuous necrotic and persistent in some cases, inconspicuous papular and fleeting in others.

Mite typhus is a disease met with in the Dutch East Indies, particularly in Sumatra. It closely resembles scrub typhus, but is nearly always characterized by the occurrence of a primary sore. Both the house rat, *Mus concolor*, and the field rat, *Mus diardii* (see Zinsser 1937), appear to constitute a reservoir of infection for the mite vector, *Trombicula deliensis*.

The viruses of these three diseases all closely resemble each other in being comparatively non-infective for guinea-pigs, in producing an inapparent infection in white rats, in causing a severe reaction in the rabbit's eye after intra-ocular injection, and in giving rise to a local necrotic lesion accompanied by fever on intradermal inoculation into the monkey. The serum of convalescent monkeys and rabbits agglutinates *Proteus OX* K but not *OX* 19. The serum of convalescent patients behaves similarly, though the serum of tsutsugamushi patients is said to agglutinate *Proteus OX* K to a lower titre than that of scrub fever or mite fever patients (Wolff 1931*a*, Kawamura *et al.* 1935). Cross-immunity experiments in the rabbit indicate the very close relationship of the three viruses to each other (see Nagayo *et al.* 1930, Kawamura and Imagawa 1931, Wolff 1931*b*, Kouwenaar and Wolff 1933, 1934, 1935, 1936, Wolff and Kouwenaar 1933, Lewthwaite and Savoor 1934, 1936*a*, *c*). Kouwenaar and Wolff (1936) are of the opinion that mite fever and tsutsugamushi can be distinguished by monkey inoculation. The observations, however, of Lewthwaite and Savoor (1936*e*, 1940*a*) seem to leave little doubt that there is complete cross-immunity between the three diseases.

If we accept this finding, we must follow Lewthwaite and Savoor (1940*b*) in giving up the terms " scrub typhus " and " mite typhus " in favour of the general term " tsutsugamushi." For a review of this disease see papers by Ogata (1931, 1935), Nagayo and his colleagues (1931), Lewthwaite (1936), and Lewthwaite and Savoor (1940*b*), and for a list of mite vectors see Williams (1944).

THE Q FEVER GROUP

Observations by workers in Australia and the United States have revealed the existence of a rickettsial tick-borne disease differing in several respects from spotted fever. Derrick (1937) described a disease, under the name of Q fever, affecting abattoir workers and dairy farmers in Queensland. The incubation period of the disease appears to be 15 days or less, the duration of illness 7 to 24 days, and the case mortality nil. Clinically, the outstanding feature of the illness is severe headache; there is no primary sore, and a rash is unusual. The Weil-Felix reaction is negative. Inoculation of guinea-pigs with the patient's blood taken during the febrile stage gives rise to a pyrexial reaction, which leaves the animal immune. Infection of rats, mice, and rabbits is of the inapparent type (Cox 1940). The virus can be demonstrated in the liver of the guinea-pig, and can be cultivated on the chorio-allantoic membrane of the developing chick embryo. Multiplication seems to occur both intra- and extra-cellularly; the nucleus of tissue cells is not, as a rule, invaded; and the virus itself can pass through Berkefeld W and N filters, but not through a Seitz disc. The natural reservoir of infection appears to be in bandicoots and opossums (Report 1938–39), among which continuity of infection is assured by infestation with the tick, *Hæmaphysalis humerosa* (Smith and Derrick 1940). Infection of man occurs indirectly. The scrub tick, *Ixodes holocyclus*, transfers infection from bandicoots to cattle, and so leads to infection of the cattle tick, *Boöphilus annulatus*, which excretes the virus in its fæces. The dried tick fæces are then inhaled by man, pneumonitis being a common primary symptom of the illness (Burnet 1942). For a description of the epidemiology of the disease in Queensland, the reader is referred to a review by Derrick (1944).

The discovery of the disease in the United States was due to Davis and Cox (1938) who, in 1935, demonstrated a filter-passing agent in wood ticks, *Dermacentor andersoni*, collected near Nine Mile Creek, Montana. Parker and Davis (1938) described its experimental transmission by the tick. Dyer (1938) reported the occurrence of infection in a laboratory worker, and later (1939) pointed out the close relationship of this disease to the Q fever of Australia. Cox (1939) suggested the name *Rickettsia diaporica* for the causative organism, but this was later abandoned in favour of *R. burneti*, which had been proposed shortly beforehand by Derrick (1939). The American virus is said to be more virulent for laboratory animals than the Australian virus, but there appears to be complete cross-immunity between the two diseases (Dyer 1939). Several laboratory infections have occurred, but whether these have been due to direct contact with infected tissues, to dust, or to the blood-sucking mite, *Lyponissus bacoti* Hirst, which infests mice, is not clear (see Burnet and Freeman 1939).

TRENCH FEVER

SYNONYMS : *Febris wolhynica : Febris quintana.*

Trench fever is a disease to which attention was first attracted in 1915, when it broke out in epidemic form amongst the troops in nearly every fighting area in Europe. Later it spread to Mesopotamia. The first account of it was given in this country by McNee, Renshaw and Brunt (1916), who called it Trench fever, and in Germany by Werner (1916) and His (1916), who called it Five-day fever and Wolhynian fever respectively. It was proved to be infective, because healthy men developed the disease when inoculated with the blood of patients suffering from it (McNee *et al.* 1916). The blood was found to be infective on the 1st day of the disease, and to remain so until at least the 51st day. Evidence was obtained that the virus was free in the blood plasma and was extra-corpuscular, but that, when the blood was allowed to clot, the virus was entangled in the fibrin and could not usually be demonstrated in the serum. Blood from trench fever patients generally gave rise to the disease in normal persons when inoculated intravenously or intramuscularly, but not when rubbed on to the scarified skin. Clinical and epidemiological evidence suggested that the disease was spread by body lice, and experimentally it was found that if lice were allowed to feed on trench fever patients and subsequently transferred to normal persons, the latter developed the disease.

Lice fed on trench fever patients do not become infective for about 5 to 9 days, but once they have become infective, they remain so for at least 4 months. The virus is found to be present in the louse's excreta, and inoculation of these excreta is one of the best methods of transmitting the disease to man—much more certain than injection of blood. By this method the War Office Trench Fever Investigation Committee (see Bruce 1921) showed that patients suffering from trench fever might retain the power of infecting lice fed on them up to at least 443 days after the onset of the disease. The infectivity of the louse's excreta is very high ; 1/10 mgm. has sufficed to give rise to the disease when inoculated into man. The virulence of the excreta is destroyed by dry heat at 100° C. in 20 minutes, and by moist heat at 60° C. in 20 minutes.

It is very doubtful whether laboratory animals are susceptible to the disease ; a small proportion of guinea-pigs may develop a low undulating type of fever after inoculation with infective material, lasting for several weeks, but infection cannot be transmitted by passage to fresh animals (da Rocha-Lima 1920*b*). On the other hand, human beings are almost all susceptible to the disease. One attack seems to produce only a partial and limited immunity ; a certain proportion of patients who have recovered from one attack may be reinfected by inoculation of blood or of louse excreta 4 or 5 months later (Bruce 1921).

In 1916 Töpfer (1916*a*) described the finding of small short rods, often arranged in pairs and often showing bipolar staining, in lice taken from trench fever patients. The rods were shorter and thicker than those seen in typhus, were present in enormous numbers, and were often arranged in heaps. Töpfer fed lice on 7 trench fever patients, and found these bodies in lice taken from 6 of them ; they did not appear till the 5th day after feeding, but from the 8th day onwards they were found in large numbers. They were never observed in lice fed on healthy persons. These bodies are now known by the name of *Rickettsia quintana.*

Töpfer's findings have been confirmed and amplified by a number of workers. Arkwright, Bacot and Duncan (1919–20) found that rickettsiæ appeared in lice 5 to 12 days after feeding on trench fever patients, and could be demonstrated in the excreta up to the death of the louse. Not all lice became infected at the same time; after feeding on a patient, only a few lice were found to be infected by the end of the 1st week; during the 2nd week the numbers infected and uninfected were about equal, while after the 2nd week the majority showed rickettsiæ. The organisms were found in large numbers crowding the region of the epithelial cells lining the gut; they were always extracellular in position. In any given batch of lice rickettsiæ developed at about the same time as the louse's excreta became infective—as judged by human inoculation. Moreover a very close correspondence was found between the presence of rickettsiæ and the infectivity of the louse, and the absence of rickettsiæ and its non-infectivity. Rickettsiæ were almost invariably found in batches of lice fed on trench fever patients, but only very rarely when fed on normal persons—one positive result out of 245 specimens.

Rickettsia quintana has never been demonstrated satisfactorily in man, and until this has been done, and until the organism has been grown in pure cell-free cultures, it will be impossible to be certain that it is the cause of trench fever. But the observations just quoted on lice strongly suggest that this organism is responsible for the disease.

The remarkable observations made on himself by Bacot (1921) lend support to this view. Bacot went to Poland in February, 1920, with the Typhus Research Commission, taking his own stock of lice with him—a stock that had been under his personal observation and fed upon himself exclusively since November, 1915. During the whole of this time rickettsiæ had never been demonstrated in these lice. In April, after visiting a bath-house in Warsaw, he contracted trench fever. Eight days later, he recommenced feeding his lice; rickettsiæ indistinguishable from *R. quintana* began to appear, and from the 12th day onwards all the boxes of lice proved to be heavily infected. Bacot's blood remained infective for at least 3 months after the clinical termination of his attack. Of the lice collected at the bath-house 7 per cent. proved to be infected with rickettsiæ, which were situated extracellularly, and which were indistinguishable from *R. quintana*. Bacot considers that these lice had probably become infected from apparently healthy persons who had some time previously suffered from trench fever. The importance of this observation lies in the interpretation to be put on the discovery of extracellular rickettsiæ in lice which have had no apparent contact with trench fever patients. Are they to be regarded as a distinct species, peculiar to the louse—as da Rocha-Lima (1920*a*) thinks—and called *R. pediculi* or *R. rocha-limæ*; or are they, in many cases, to be regarded as *R. quintana*, which has gained access to the louse from probably healthy carriers of trench fever? Bacot clearly favours the latter view.

A few cases of trench fever were recognized after the war of 1914–18, and there is reason to believe that the disease is endemic in Poland and Russia (Laurell 1933, Braslawsky 1934). During the second world war numerous outbreaks were reported in the German army on the eastern front, but little new was learnt about the ætiology of the disease (see Marie 1943). The reaction of patients' sera in the Weil-Felix test is still doubtful.

Classification of the Rickettsial Diseases of Man.

Attempts have been made to classify the rickettsial diseases on the nature of the transmitting vector (Megaw 1921, 1930), and on the animal reservoir of infection (Megaw 1942). Besides breaking down at a number of points, such classifications are unsuitable for bacteriological purposes. Felix (1935, 1942*a*) believes that a satisfactory classification can be based only on the antigenic type of the

causative organism, upon which immunity and immunity reactions depend. On the whole, we are in general agreement with this proposal, but we consider it desirable to take into consideration other properties than the antigenic structure alone, such as the morphology of the different viruses, their mode of growth in tissue culture, their infectivity for man and animals, and the type of disease and the pathological lesions to which they give rise. Unfortunately our knowledge is still too fragmentary to enable us to make more than a tentative subdivision on this basis, but the scheme given in Table 182 may serve to indicate the main lines which such a classification should probably follow.

Within the four main groups—excluding trench fever, about which our knowledge is very scanty—there are sub-groups often associated with a particular host, vector or locality. The corresponding viruses tend to differ, particularly in their degree, of pathogenicity for man and laboratory animals. So far, experimental workers have not been successful in converting one sub-type into another, though Mooser, Varela, and Pilz (1934) and Mooser (1941*a*) seem to regard the classical and murine types of the typhus virus as interchangeable.

Diagnosis.

Since the demonstration of the causative organism by growth in tissue culture, the developing chick embryo, or the louse's gut is a lengthy procedure, possible only under favourable conditions, recourse is had mainly to an agglutination test on the patient's serum. The only diagnostic test of importance is the Weil-Felix reaction. In 1916 Weil and Felix, working in Eastern Galicia, cultivated from the urine of a patient with typhus a proteus-like organism that had the peculiarity of being agglutinated not only by the patient's serum but also by the sera of other patients suffering from typhus. Similar organisms were isolated from the urine or the blood of a number of other typhus patients; these were called *Proteus X* strains; the most highly agglutinable of them, and the most specific for typhus, was the *Proteus X* 19 strain (Felix 1916). This strain was agglutinated to a titre varying from 1/50 to 1/50,000 by typhus sera, and never above 1/25 by serum from non-typhus patients. Agglutinins appeared in the blood about the 4th day, and increased up to an acme about the end of the second or beginning of the 3rd week; during convalescence they fell off rapidly, and after 5 months they had disappeared almost entirely. A positive reaction at 1/25 during the 1st week of the disease was regarded as a presumptive positive, though about 7–9 per cent. of persons not suffering from typhus were found to react at this titre. As a rule the titre rose rapidly, so that a positive diagnosis, 1/50 or higher, could generally be made by the 8th day.

The Weil-Felix reaction has now been employed on a large scale, and has been found remarkably valuable. As with many other serum reactions used in diagnosis, experience of it has brought to light certain anomalies and limitations that were not at first realized. Many workers have doubted its specificity, and have brought evidence to show that the reaction may be positive during the course of other infections, and even in healthy persons.

Savoor, Castaneda, and Zinsser (1935), for example, in the United States, found that 25 out of 596 sera from various sources reacted positively in a titre of 1/80, and 4 in a titre of 1/160 (see also Peverelli 1930, Kraus *et al.* 1931, Isabolinski *et al.* 1934, Welch *et al.* 1934). In some of these instances it is probable that the patient had recently recovered from typhus fever, or was suffering from it in a mild or latent form. Sonnenschein (1943) has recorded titres of 1/200 to 1/1600 to *Proteus OX* 19 in patients suffering from *Proteus*

TABLE 182

TENTATIVE CLASSIFICATION OF THE RICKETTSIAL DISEASES OF MAN (Modified from Felix 1942*a*).

Group.	Typhus	Spotted Fever.	Tsutsugamushi	Q Fever.	Trench Fever
NAME OF DISEASE	Classical epidemic typhus. Mexican typhus (Tabardillo). Brill's disease. Endemic typhus of U.S.A., Australia, Greece, Syria, Manchuria, etc. Shop typhus of Malaya. Fièvre nautique of Toulon.	Rocky Mountain spotted fever. Brazilian spotted fever (São Paulo typhus). Fièvre boutonneuse. Kenya fever. Tick-borne fever of South Africa, and probably of India.	Tsutsugamushi. Scrub typhus of Malaya. Mite typhus of Dutch East Indies.	Q fever of Queensland. Q fever of North America.	Trench fever. (Wolhynian fever, Febris quintana).
VECTOR . .	Lice and rat fleas.	Ticks.	Mites.	Ticks.	Lice.
RESERVOIR OF VIRUS.	Rats. Man.	Rodents. Dogs. Sheep.	Voles. Wild rats.	Bandicoots. Opossums. Dogs. Cattle.	? Man.
VIRUS . .	*R. prowazeki*; *R. mooseri*. Non-filtrable. Grows in the cytoplasm. No extracellular growth.	*R. rickettsi*; *R. rickettsi* var. *conori*; *R. rickettsi* var. *pijperi*. Non-filtrable. Grows mainly in the nucleus. No extracellular growth.	*R. nipponica*. Non-filtrable. Grows only in the cytoplasm. No extracellular growth.	*R. burneti*. Filtrable. Grows mainly in the cytoplasm. Some extracellular growth.	*R. quintana*. Non-filtrable. Not yet cultivated *in vitro*.
AGGLUTINATION	*OX* 19 +++. *OX* 2 +. *OX* K −	*OX* 19 +. *OX* 2 +. *OX* K +	*OX* 19 −. *OX* 2 −. *OX* K +++	*OX* 19 −. *OX* 2 −. *OX* K −	?
SUSCEPTIBLE ANIMALS	Febrile reaction in guinea-pigs; scrotal reaction with *mooseri* variety. Inapparent infection in rats. Classical variety dies out in mice. Rabbits insusceptible. Cross-immunity between classical and murine types probably not quite complete. Some degree of cross-immunity between typhus fever and spotted fever.	Usually severe disease in guinea-pigs, often with hæmorrhagic necrosis of scrotum and ears. Febrile disease in rabbits. Inapparent disease in mice and rats. Cross-immunity complete between Rocky Mountain and Brazilian spotted fever, and fièvre boutonneuse; incomplete with South African tick-borne fever.	Relatively non-infective for guinea-pigs, but may produce ascites. Acute reaction after intra-ocular injection of rabbits. Rats and mice difficult to infect. Cross-immunity complete between all three diseases.	Febrile disease in guinea-pigs, but no scrotal reaction. Inapparent infection in mice. Rabbits insusceptible. Cross-immunity between the two diseases complete.	Not transmissible to laboratory animals.

infections such as pyelitis, cystitis, cholecystitis, pleuritis, and bronchitis, as well as in patients with *Proteus*-infected wounds; and Smith and Evans (1943) have noted a raised and fluctuating titre in the course of other diseases like undulant fever, enteritis, typhoid fever, and dysentery (see also de Moor 1936, de Roda 1937).

It follows that the result of the Weil-Felix test should be interpreted strictly in relation to the clinical findings. Generally speaking, it may be said that, except in endemic areas or in patients who have previously suffered from typhus fever or been inoculated against it, complete agglutination of *OX* 19 in a final dilution of the patient's serum of 1/100 or over during the second week of the disease is highly suggestive of active infection. Cases, however, do occur in which the antibody response is very poor or transient, and in which the result of the test has to be regarded as doubtful or negative. Repeated examinations at intervals of 3 or 4 days are of value in detecting transitory reactions and changes in titre indicative of active infection. A strong reaction is regarded by Maly (1942) as a good prognostic sign; a positive reaction becoming negative during the course of the disease as indicative of severe toxæmia and a fatal outcome. According to Felix (1944), however, a low titre may occur not only in very severe, but also in very mild cases. In murine typhus the titres tend to be lower than in the classical form (Findlay 1941). Diagnosis in an inoculated patient does not differ materially from that in an uninoculated patient. Inoculation with the chick embryo vaccine is said not to stimulate agglutinins for *Proteus OX* 19 (Report 1945). If residual agglutinins are present as the result of inoculation with other types of vaccine, they are usually present in very low titre. A rising titre for *OX* 19 therefore is highly suggestive of active infection (Felix 1944).

It is important to realize that agglutination of the O antigen alone of *Proteus* is indicative of typhus infection. Normal agglutinins to the H antigen of *Proteus* strains are not uncommon and have no significance in the diagnosis of typhus fever. In practice, it is therefore wise to work with suspensions prepared from a non-motile *Proteus X* 19 strain, so as to avoid confusion caused by flagellar agglutinins. For this purpose, the formolized suspensions described by Bridges (1935), and issued by the Oxford Standards Laboratory, are most suitable. Agglutination of a non-motile strain, or agglutination of the small flake type of a motile strain, is best referred to as agglutination of *Proteus OX* 19. The test itself is usually carried out by the tube method, but for rapid diagnosis and in epidemiological surveys the slide agglutination method may be used (see Eyer and Grützner 1940, Steuer 1942). Pure suspensions of rickettsiæ, prepared from the lungs of white rats or mice or in some other way, may be used instead of *Proteus OX* 19 (see Hudson 1940), but seem to present little advantage for ordinary routine work.

So far we have been considering the diagnosis only of the typhus group of fevers by the Weil-Felix test. A modified form of the test is, however, of value in the diagnosis of tsutsugamushi and of the spotted fever group.

Fletcher and Lesslar (1925, 1926), studying the endemic typhus of the Federated Malay States, observed that the Weil-Felix reaction to *Proteus OX* 19 was positive in some cases but not in others. Wondering whether their strain of *Proteus X* 19 was losing its agglutinability, they tried a fresh strain that had been brought out to the Straits Settlements by Dr. Kingsbury from the Middlesex Hospital. This strain, which may be referred to as *Proteus X* K, was found to be agglutinated by the sera of patients which failed to react with the *X* 19 type. It was further

observed that sera which reacted to *OX* 19 did not agglutinate *OX* K. There was clearly some distinct serological difference between the two groups of typhus cases. The Kingsbury strain, it may be noted, was reputed to be a strain of *X* 19, but undoubtedly differed from this type antigenically. Further work (see also Fletcher 1930, 1932) showed that the typhus patients whose blood agglutinated *OX* 19 were to be found mainly in urban areas, particularly in shops and storehouses, while those whose blood agglutinated *OX* K came from country districts, especially places where rank grass and scrub had grown up on land which had been cleared of jungle. Fletcher referred to the former type of typhus as urban or *shop typhus*, and to the latter as rural or *scrub typhus*.

We have already described how the urban or shop typhus is carried by rats and is of the ordinary endemic murine type, whereas rural or scrub typhus appears to be identical with the tsutsugamushi disease of Japan. Further experience has confirmed the value of the agglutination of *OX* K in the diagnosis of the tsutsugamushi group of fevers. *Proteus OX* K tends to be agglutinated more often and to a rather higher titre by normal sera than *OX* 19 (see Report 1942), so that complete agglutination in a final dilution of the patient's serum of 1/200 should be demanded for diagnostic purposes. Even then, great care must be exercised in interpreting the result.

A strain of *Proteus X* corresponding to the virus of the spotted fever group has not yet been discovered, but a reaction, often not appearing till late in the second week, may occur with one or more of the *Proteus OX* 19, *OX* 2, or *OX* K strains. Davis, Parker and Walker (1934) found that 77 per cent. of sera taken between the 10th and 32nd days of illness agglutinated *Proteus OX* 19 to 1/160 or over; when two samples were taken at suitable intervals, 95 per cent. of sera proved positive. According to Davis and Parker (1938) a relatively high *OX* 19 and a relatively low *OX* 2 titre is the most frequent combination found. Such a result, or agglutination of *OX* 19 alone, may be due to typhus fever; on the other hand, a high *OX* 2 titre, either alone or in combination with a low *OX* 19 titre, is suggestive of spotted fever (see Felix 1942*b*). *Proteus OX* K is usually agglutinated least of all.

In Q fever the Weil-Felix reaction appears to be completely negative, though agglutination of pure rickettsial suspensions, to a variable titre, may be observed in infected patients (Burnet and Freeman 1938).

For a description of the technique and interpretation of the Weil-Felix reaction in different rickettsial diseases, an article by Felix (1944) may be consulted.

A few reports have appeared on the use of the *complement-fixation reaction* in the diagnosis of rickettsial diseases. Castaneda (1936) demonstrated the presence of specific complement-fixing bodies in the serum of typhus patients; and Bengtson (1941) and Bengtson and Topping (1941) confirmed their specificity by showing that, with an antigen prepared from *R. mooseri*, no reaction was obtained with serum from patients suffering from other diseases, including Rocky Mountain spotted fever and Q fever. Plotz and Wertman (1942), using an antigen prepared from *R. rickettsi*, reported positive results with spotted fever but not with typhus sera. It appears, therefore, that the complement-fixation test tends to be more specific than the Weil-Felix reaction. It has, according to Bengtson and Topping (1942), the additional advantage of enabling a retrospective diagnosis to be made more readily, since the fall in complement-fixing bodies is slower than that of *Proteus OX* agglutinating bodies. For the same reason it is said to be of particular

value in the diagnosis of infection of rats with *R. mooseri* (Brigham and Bengtson 1945).

Other methods of diagnosis may sometimes have to be employed. In Rocky Mountain spotted fever, for example, it may be necessary, if the result of the Weil-Felix test is ambiguous, to resort to the so-called *infection test.* This consists in inoculating the patient's blood intraperitoneally into a male guinea-pig to see whether the typical scrotal reaction of swelling, reddening, and sloughing occurs. In severe cases of the disease this test is often positive, but in mild cases little attention can be paid to a negative result. During convalescence the *virus neutralization test,* in which a mixture of *R. rickettsi* and the patient's serum is injected intraperitoneally into guinea-pigs, together with suitable controls, is more likely to be of value. A similar test for the retrospective diagnosis of typhus fever, in which the mixture of virus and serum is injected into the skin of the rabbit, has been described by Giroud (1938), and found by Clavero and Gallardo (1942) to be specific.

Discussion of the Weil-Felix Reaction.

The explanation of this reaction is still obscure.

That the *Proteus X* bacilli are not themselves responsible for the disease is indicated by the following facts: (1) they are found in only a minority of typhus patients; (2) they do not produce experimental typhus in the guinea-pig; (3) they are never isolated from guinea-pigs infected with true typhus virus; (4) guinea-pigs inoculated with *Proteus X* bacilli develop no immunity to the subsequent inoculation of typhus virus; (5) guinea-pigs that have recovered after inoculation of typhus virus are as susceptible as normal guinea-pigs to a lethal dose of *Proteus X* bacilli (Otto 1919).

Felix himself is of the opinion that the two organisms are genetically related and that *Proteus X* must be regarded as a variant of *Rickettsia*, differing from this organism in its morphological, cultural, and pathogenic properties, but resembling it in the possession of a common O antigen (see Chapter 39 and Felix and Rhodes 1931, Felix 1933*a*, *b*, 1935). If this is so, then *Proteus X* 19 may be looked upon as the variant of *R. prowazeki*, and *Proteus X* K as the variant of the *Rickettsia* of scrub typhus. *Proteus X* variants corresponding to *R. rickettsi*, *R. burneti*, and *R. quintana* have not yet been discovered, though it is interesting to note that strains with a dual antigenic structure responding to both *OX* 19 and *OX* K agglutinins have been reported in Tunis (type *S* 24) and in São Paulo (type *X* L) (see Laigret and Durand 1934).

We should be unwise, however, to accept such an explanation without definite proof. Clearly, the most satisfactory evidence would be afforded by a direct demonstration of the change from *Rickettsia* to *Proteus*, either in tissue culture or in the louse's intestine. Attempts to prove the existence of a genetic relationship along these lines have not yet proved successful, though suggestive results have been recorded by Weigl (1923), Anigstein (1933), and Anigstein and Lawkowicz (1939).

As a corollary it would be important to show that *Proteus X* strains were never encountered except in relation to *Rickettsia.* Our available information on this point is not very reassuring.

A number of workers (see Sparrow and Roussel 1936, 1937, van Loghem 1936) have isolated *Proteus X* 19 strains from persons suffering from diseases other than typhus. Sparrow and Roussel, working in Tunis where typhus is epidemic, cultivated organisms of the *X* 19 type from the blood of about 50 per cent. of typhus patients in 1935, and from about 9 per cent. of patients suffering from other diseases, such as undulant fever,

typhoid fever, malaria, and pulmonary tuberculosis; in the following year, however, the proportion of positive cultures was very much lower. It may further be noted that *Proteus* strains have been found in the blood and urine of typhus fever patients which were not of the *X* 19 type (Schürer and Wolff 1919; see also Felix 1931).

Some workers, such as Otto (1919), have regarded the Weil-Felix reaction as an example of para-agglutination, using this term to mean the agglutination of a heterologous organism by virtue of a property conferred on it by contact with the homologous organism. It is supposed that *Proteus* bacilli in the body of a typhus-infected patient gain new antigenic receptors, which render them susceptible to antibodies produced against the typhus virus. If this were true, it would explain the origin of *Proteus X* types corresponding to different rickettsial types; but insufficient evidence has yet been adduced to render this more than a speculation.

A more or less similar conception has been advanced by W. J. Wilson (1909, 1920), who showed that certain coliform bacilli isolated from the urine or fæces of typhus patients were agglutinated more readily by typhus sera than by normal sera. Wilson believes that under the stimulus of infection with the typhus virus, some normal intestinal bacterium calls forth the production of specific agglutinins to itself and of group agglutinins to other organisms, such as *Proteus X* 19. This suggestion receives some support from the observations of Giroud and Tannenbaum (1937). Trying to solve the problem why infected guinea-pigs, unlike rabbits and monkeys, do not give a positive Weil-Felix reaction, these workers observed that *Proteus* bacilli are never found in the guinea-pig's intestine. They therefore infected a group of these animals with typhus virus parenterally and with *Proteus* bacilli by the mouth, and found that their serum developed the property of agglutinating *Proteus OX* 19. Incidentally, it may be noted that, though the serum of typhus-infected guinea-pigs does not agglutinate *Proteus X* 19, it does agglutinate pure suspensions of rickettsiæ (Hudson 1940).

An alternative explanation is that the Weil-Felix reaction is an example of group agglutination (see Fairley 1919–20). It is known (see p. 933) that *Proteus X* 19 and *R. prowazeki* share a common antigen, and so far as these two organisms are concerned, such an explanation is satisfactory. The existence, however, of a similar relationship between *Proteus X* K and *R. nipponica* necessitates the assumption that an antigenic variety of one genus is reflected in a corresponding variety within the other genus. This has been regarded, perhaps unjustifiably, as too much of a coincidence to be probable. It would, perhaps, be less probable if *Proteus X* strains corresponding to *R. rickettsi*, *R. burneti*, and *R. quintana* were known to exist; but so far attempts to find these variants have proved a failure. We are therefore left at present with only two *Proteus X* types corresponding to two rickettsial types, depending, in the case of *R. prowazeki*, upon a similarity in the chemical structure of its glycolipoid antigen with that of the glycolipoid antigen of *Proteus X* 19 (Ciuca *et al.* 1938).

Prophylaxis and Treatment of the Typhus Fevers.

As in other diseases which are purely insect-borne, the prevention of rickettsial infections resolves itself into the control or elimination of the insect vector. In the particular instance of epidemic typhus fever the essential factor is the prevention of the frequent passage of the body louse from man to man; and this is best attained by reducing louse infestation to a minimum, and by the prevention of overcrowding. Typhus is not a disease of which any cleanly and reasonably

prosperous community need stand in dread during times of peace ; its whole history shows its close relation to poverty, malnutrition, overcrowding, dirt, wretchedness, and war. The control of endemic typhus fevers presents a more difficult problem, partly because of the widespread distribution of the various infecting agents, and partly because of the sporadic nature of the disease in human beings. Destruction of the normal animal reservoir of infection, such as the rat, may be more effective than attempts to interfere with the passage of the insect vector to man, though these will generally have to be made in addition. For a description of the hygienic measures required to control louse-borne typhus fever the reader is referred to a paper by Mackenzie (1941) and to a memorandum by the Ministry of Health (Memo. 1941). Neither of these publications, however, makes mention of the extremely effective methods that were introduced during the second world war for the prevention of louse infestation by impregnating the clothing with certain chemical substances. Under some conditions, precautions may have to be taken to prevent infection by the respiratory tract with dried insect fæces or other infective forms of dust.

Chemotherapy has not yet proved of value in typhus fever. Experimentally, Andrewes and his colleagues (1944) have reported favourable results in mice infected with the murine or the classical typhus virus by the use of two sulphamido compounds, but these have apparently no effect on man. Similarly, Greiff, Pinkerton and Moragues (1944) find that both penicillin and para-aminobenzoic acid inhibit growth of the murine virus in the yolk sac of the chick embryo ; human trials with these substances are in progress.

Vaccination.

Attempts to produce active immunity by vaccination have been made by a number of workers. Without reference to chronological order, these may be classified as follows.

Living Vaccines.—Nicolle and Laigret (1936) prepared a vaccine of murine virus from infected guinea-pig and rat brain. The organisms were dried, coated with egg yolk, dried again, and finally suspended in olive oil. Three doses were given. The vaccine has been tried on several thousand persons, mainly natives, with apparently favourable results (Laigret *et al.* 1937). A few cases of inoculation typhus have resulted.

Killed Vaccines.—In preparing a dead vaccine, the problem is how to obtain a thick suspension of rickettsiæ. The following solutions have been proposed.

(*a*) *Weigl's louse vaccine.*—Lice are inoculated by the rectum with the classical typhus virus. After a week or so they are killed ; the intestines are removed and ground up in 0·5 per cent. phenolized saline (Weigl 1933). This vaccine has been used on a fairly large scale and appears to have met with considerable success (see Radlo 1937, Mosing 1938). Since three doses are given, requiring the use of 120–175 lice for each subject, an enormous louse farm has to be maintained if vaccine is to be prepared on any considerable scale. An additional drawback is the necessity for feeding the lice for a week after infection on immune persons.

(*b*) *Killed suspensions of rickettsiæ grown in animals.*—Various methods have been used. (1) Zinsser and Castaneda (1930*a*, 1931, 1933) introduced the intraperitoneal inoculation with a murine virus of guinea-pigs or rats whose resistance had been lowered by injections of benzol, feeding on a vitamin-deficient diet, or best of all irradiation with X-rays (see also Macchiavello and Dresser 1935). In such animals great proliferation occurred in the peritoneum and tunica vaginalis ;

a thick rickettsial suspension could be obtained by washing out the peritoneal cavity, and converted into a vaccine by treatment with 0·2 per cent. formol. This vaccine has had only a limited trial, but according to reports by Veintemillas (1939) in Bolivia and by Finlayson and Grobler (1940*a*, *b*, 1941) in South Africa, it seems to protect not only against the murine virus, but also—provided a large enough dose is given—against the classical typhus virus. (2) Castaneda (1939) found that the lungs of rats which had been inoculated intranasally with a murine virus contained abundant rickettsiæ. A vaccine was prepared by grinding up the lungs of these animals 3 days after inoculation, killing the rickettsiæ with phenol, and obtaining a suspension of the organisms by differential centrifugation. Three doses of vaccine are recommended for man at 7-day intervals (Castaneda 1941). Guinea-pig experiments show that this vaccine protects readily against infection with the murine virus, but only when given in large dosage against the classical virus. (3) Okamoto (1937) observed that rickettsiæ of the murine type were able to multiply in the mouse's lung. Durand and Sparrow (1940) made use of this observation, and found that mice inoculated intranasally with the murine or the classical typhus virus died in 3 to 7 days, and that at post mortem their lungs contained enormous numbers of rickettsiæ. Durand and Giroud (1940*a*) and Giroud and Panthier (1942) prepared a vaccine from a formolized suspension of ground-up mouse lung, and found it to protect guinea-pigs against experimental infection. For human beings 4 or 5 weekly inoculations appear to be required. Rabbit lung and dog lung vaccines have also been used.

(*c*) *Killed suspensions of rickettsiæ grown in tissue culture.*—Zinsser, Fitzpatrick and Wei (1939) have described a relatively simple method of growing rickettsiæ in a modified Maitland medium making use of agar blocks covered with living tissue. The growth can be removed and treated with 0·3 per cent. formol for conversion into a vaccine.

(*d*) *Chick embryo vaccine.*—Cox (1938, 1941) found that much the best method of growing rickettsiæ of typhus fever, spotted fever and Q fever was in the yolk sac of the developing chick embryo. Once strains of the classical typhus virus are established, they usually kill the embryo in 3 to 5 days. The pooled embryonic tissues are then ground up and treated with phenol and formalin. Yolk sac suspensions are 100 to 1,000 times more infective than suspensions of mammalian tissues. The method is very simple and applicable to large-scale production. This vaccine has achieved wide popularity, and was used in the second world war for the inoculation of the British and American forces.

Insufficient controlled observations have as yet been made to enable any sound conclusions to be drawn on the absolute and relative protective value of these different vaccines. Otto and Bickhardt (1942) found mouse and rabbit lung vaccines to be superior to chick embryo vaccines in the protection of guinea-pigs. Ding (1943), on the other hand, who made observations on human beings under what seem to have been almost experimental conditions, was led to conclude that rabbit and dog lung vaccines and chick embryo vaccines were equally satisfactory and yielded results not inferior to those of Weigl's louse vaccine. None of them appeared to protect against the disease itself, but all of them shortened the fever, diminished the nervous symptoms, rendered the illness milder, and conferred a high degree of protection against death. Plenty of other evidence is available to show that vaccination does not prevent an attack of the disease, at any rate if exposure to infection is severe (see Löffler and Mooser 1942, van

den Ende *et al.* 1943). American observations suggest that, judged by animal experiments, the Castaneda rat lung and the Cox chick embryo vaccines are both inferior to Giroud's mouse lung vaccine. Chick embryo vaccine can be improved by the addition of alum (Report 1945). How far a murine typhus vaccine is effective against the classical virus is still in doubt. According to Mooser and Leemann (1941) there is in animals a complete cross-immunity between the two diseases; but there are numerous published and unpublished observations suggesting that this does not hold for man. The question, however, is difficult to answer, since even a vaccine prepared with one type does not afford complete protection against inoculation with the same type. Whatever vaccine is used, repeated single doses at 6-monthly intervals to stimulate the waning immunity are desirable if the risk of exposure to infection continues. (For a description of the mode of preparation of the different vaccines, see Clavero and Gallardo 1943, and for a study of the chick embryo vaccine, in particular, see Report 1945.)

Similar vaccines have been used for inoculation against the spotted fever group. Indeed it was against Rocky Mountain spotted fever that a satisfactory killed vaccine was first produced by Spencer and Parker (1925), when they prepared a phenolized suspension of the viscera of infected ticks. A formolized mouse lung vaccine has been described by Durand and Giroud (1940*b*). Protection of experimental animals against Q fever may be obtained by vaccines prepared from chick embryo tissues or from the fæces of *Dermacentor andersoni* (Cox 1940). Rickettsial suspensions prepared from infected mouse spleens and killed by 0·5 per cent. formol or by heating at 60°–65° C. for 30 minutes are also said to be effective (Burnet and Freeman 1941). Against scrub typhus a vaccine has been prepared by Fulton, but no account of it has yet been published.

Serum Treatment.

Nicolle, Conor and Conseil (1911) found that the serum of human patients or of monkeys convalescent from typhus fever possessed protective and curative properties, when tested on experimental infection in monkeys or the natural disease in man. The serum was most active on the 10th to the 12th day after defervescence; after the 15th to the 20th day it had lost its activity completely, even though the monkey itself was resistant to a fresh inoculation of virus. Nicolle introduced the treatment of the disease by such serum. For *prophylactic* purposes he injected about 3 doses of blood serum, obtained by centrifugalization of the coagulated blood. According to his observations, persons treated by this method subsequently proved resistant to the inoculation of 3 ml. of blood from a typhus guinea-pig. For *therapeutic* purposes the blood serum of convalescent patients may be injected in fairly large quantities. Little is as yet known of the value of this method of treatment.

More recently, Durand and Balozet (1940, 1941) have prepared antiserum by the inoculation of horses and asses with mouse lung suspensions of the murine and classical types, and by animal experiments have found it to be considerably more potent than convalescent human serum. The neutralizing power does not run parallel with the Weil-Felix agglutination titre, since the antigen responsible for the stimulation of neutralizing antibody appears to be the heat-labile, not the heat-stable, rickettsial antigen. In practice, this serum is given therapeutically in a single dose of 100 ml. intramuscularly. The few observations that have so far been made on it suggest that, if given early, it shortens the duration of the illness and diminishes the risk of death (see Wolman 1944). Similar results

have been claimed by Topping (1943) for the treatment of Rocky Mountain spotted fever by a serum prepared by the inoculation of rabbits with infected ticks or yolk sac cultures.

Rickettsial Infection of Animals

Though animals often act as a reservoir of rickettsiæ that are pathogenic for man, little is known as yet of the occurrence of animal disease due to other types. The best example is heartwater.

Heartwater.—Economically, this is an important disease of sheep, goats, and cattle. It is widespread over the whole of Africa, except the northern portion. Infection is due to *Rickettsia ruminantium*, first described by Cowdry (1925), and is transmitted by the tick *Amblyomma hebræum*. Clinically, the disease varies from the hyperacute form, on the one hand, to the abortive form, on the other. The incubation period in sheep is 7 to 14 days. The acute form is characterized by fever, serous effusions in the pleura, pericardium, and peritoneum, and convulsive symptoms. The course is 2–6 days, and the case mortality 50–100 per cent. Cases occur sporadically and in small groups, not in large epidemics (Donatien and Lestoquard 1937). *R. ruminantium*, like the organisms of typhus and of spotted fever, infects primarily the vascular endothelium, and like *R. prowazeki*, but unlike *R. rickettsi*, grows in the cytoplasm, not in the nucleus of the cells. Laboratory animals seem to be relatively insusceptible to infection. No satisfactory method of vaccination against the disease has yet been described, but the disease is said to be susceptible to sulphonamide treatment (Neitz 1940). Some workers regard heartwater as a virus rather than a rickettsial infection and would classify it in the lymphogranuloma-psittacosis-virus-pneumonia group of diseases. The evidence is still inconclusive, and we consider it wiser for the time being to retain it in this chapter.

Other Infections.—Mention has already been made in the chapter on *Rickettsia* (pp. 936–7) of organisms described under the term *R. conjunctivæ* which appear to infect the conjunctival epithelial cells of certain animals, and of organisms described as *R. canis*, *R. ovina*, and *R. bovis* affecting the monocytes. The claim of these organisms to belong to the rickettsial group is still unsubstantiated, and since, with the exception of *R. canis*, the diseases with which they are associated are very benign, we shall deal with them no further.

For a general description of the rickettsial diseases, the reader is referred to reviews by Zinsser (1937), Parker (1938), Lewthwaite and Savoor (1940*b*), and Wolbach (1941).

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CHAPTER 84

PLEUROPNEUMONIA, CONTAGIOUS AGALACTIA, AND ALLIED DISEASES

PLEUROPNEUMONIA OF CATTLE

PLEUROPNEUMONIA is an infectious disease of cattle characterized by an exudative inflammation of the interlobular lymph vessels and of the alveolar tissue of the lungs, with a simultaneous sero-fibrinous pleurisy. According to Nocard and Roux (1898), the essential lesion is a distension of the connective tissue layers between the lobules of the lungs with a large quantity of yellowish, limpid, albuminous, highly infective fluid. The disease is widespread throughout the world, with the exception of Western Europe, India, and North America (Walker 1930). It used to be common in Europe, but preventive measures have greatly decreased its incidence, while in the United States it has been completely eradicated. Epizootics are common in endemic areas; the morbidity is high; the case mortality varies considerably according to the severity of the disease. The mode of infection is not clear, but there is reason to believe that it is carried by droplets, and indirectly through dust particles contaminated with nasal discharge (Tang *et al.* 1935). The causative organism of the disease is characterized by a high degree of pleomorphism and by its filtrability. The disease can be reproduced experimentally by inoculation of cows with infective material or cultures of the filtrable organism (see Chapter 40). Active immunization is practised by inoculation of tissue fluid, or preferably of a living culture of the organism, into the point of the tail. This results, as a rule, in a non-fatal disease, which leaves the animal immune for a year or more. Most workers recommend a strain of modified virulence (Bennett 1932, Mettam and Ford 1939, Purchase 1939), but Kurotchkin (1939) prefers a fully virulent strain. The serum of convalescent animals is used for passive immunization. Not all strains appear to be identical, and it is possible that an animal that has recovered from one attack may prove susceptible to later invasion by a different strain. In practice the slaughter policy is recommended for the control of the disease. In carrying out this policy, the complement-fixation test may be of value in the detection of carriers. According to Bennetts (1937), the test itself is reliable, but the eradication method based on it is not always successful, owing to the long incubation period of the disease and other causes. (For pleuropneumonia of calves due to *Pasteurella septica*, see p. 1647.)

CONTAGIOUS AGALACTIA OF SHEEP AND GOATS

This disease is characterized by inflammatory lesions of the udder, eye, and joints. The milk yield in lactating animals is diminished, and instead of milk, a

dirty yellow serous fluid containing small clots is secreted (Galloway 1930). Several outbreaks have occurred during the past hundred years in Europe, and more recently the disease has been encountered in North Africa. The case mortality is said to average about 15 per cent. The causative organism, which was first cultivated by Bridré and Donatien (1923, 1925), resembles the organism of pleuropneumonia in its pleomorphism and its filtrability. Natural infection usually appears to occur by ingestion. The disease can be reproduced by inoculation, especially of goats, with infected tissue suspensions, and with pure cultures (see Chapter 40). Animals that have recovered naturally from an attack of the disease have a high degree of immunity. Active immunization is very difficult. Passive immunity may be conferred by the injection of immune sera prepared in sheep, goats, or horses (Bridré and Donatien 1925).

DISEASES CAUSED BY OTHER MEMBERS OF THE PLEUROPNEUMONIA GROUP OF ORGANISMS

Numerous diseases of animals have now been ascribed to infection with other members of the pleuropneumonia group, such as arthritis, lymphadenitis, and suppurative lesions in rats, and arthritis and rolling disease in mice. The relation of these organisms to bronchiectasis in rats, distemper in dogs, and certain genital fesions in the human female is still under discussion. Space does not allow a lull description of each of these diseases; adequate attention has already been paid to them in Chapter 40, which the reader may consult for references to the original papers.

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CHAPTER 85

FILTRABLE VIRUS DISEASES

A. THE LYMPHOGRANULOMA-PSITTACOSIS GROUP

In this and the following four chapters we describe the most important diseases of man and animals that are caused, or probably caused, by filtrable viruses. We are immediately faced with the problem of their classification. Numerous suggestions have been made by different workers for grouping these diseases according to the tissue attacked, the nature of the lesion produced, the presence of inclusion bodies, the transmissibility of infection, and so on (see Rivers 1932, Fine 1932, McKinley 1934, Fairbrother 1934, Hurst 1936). It is clear that the only satisfactory classification must rest on the nature and properties of the causative viruses themselves. Our knowledge, however, of the viruses is extremely meagre, and though tentative efforts at their classification are doubtless of value in research work, it is perhaps a little premature to reproduce them in what is primarily a textbook designed for teaching. We shall, therefore, omit any serious attempt at classification. The order in which we shall describe the various diseases will be determined partly by their public health importance, and partly by the nature of the organs or tissues mainly attacked. It may be noted that influenza and swine influenza have already been dealt with in the chapter on respiratory infections (Chapter 74).

The viruses described in this chapter fall into a group that appears to be intermediate between the rickettsiæ and the true filtrable viruses. Most or all of them form elementary bodies that take on basic stains and can be readily seen under the microscope; some of them are known to pass through a series of morphological changes during growth; and most of them appear to be closely related antigenically. They differ from the rickettsiæ in being spherical rather than rod-shaped, in having no antigenic affinity to *Proteus OX* strains, and in the absence of an insect host. They differ from the common filtrable viruses in their larger size—usually about 150–300 mμ—in being held back partly or completely by a Berkefeld N candle or a Seitz disc, and in their failure, as a rule, to give rise to a lasting immunity. Some of them are distinguished by their reputed susceptibility to the sulphonamide drugs.

LYMPHOGRANULOMA INGUINALE

Synonyms: Climatic bubo; Lymphopathia venereum; Poradenitis. Not to be confused with Granuloma venereum (see p. 1790).

In 1913 Durand, Nicolas and Favre of Lyons drew attention to a bubonic disease, probably of venereal origin, which was characterized by a subacute inflam-

mation of the inguinal lymphatic glands. In 1925 Frei reported the discovery of a specific intracutaneous test for the diagnosis of the disease. As a result of this means of differentiating lymphogranuloma from other diseases, several workers entered the field of investigation. In 1930 Hellerström and Wassén at the International Dermatological Congress in Copenhagen described the successful transmission of infection to monkeys by intracerebral inoculation, and concluded that the disease was of virus origin. Since then there has been a large outcrop of papers on various aspects of the subject, which it is impossible to summarize adequately in the space at our disposal. Those who are desirous of more detailed information are referred to monographs and papers by Stannus (1932), Koch (1933), Findlay (1933), Hellerström and Wassén (1934), Wassén (1935), Levaditi, Mollaret and Reinié (1935), and Nelson (1941).

Though showing a preference for the tropics, the disease is widespread throughout the world. Males of 20–40 years of age are most often affected. The disease is contracted through sexual intercourse. After healing of the initial penile lesion, males cease to be infectious, whereas females, on account of the peculiar lymphatic distribution in the perineal region, remain infectious for months and years. In females particularly, the disease often involves the genito-ano-rectal tissues with the production of a stricture—a condition referred to as *esthiomène*—though it is not yet established that all cases of esthiomène are caused by the lymphogranuloma virus.

Attention was first concentrated on the local lesions, but the disease is now known to be accompanied by a generalized infection. In its early stages it may even simulate typhoid fever; arthritis and skin rashes are not uncommon, and symptoms suggestive of meningeal affection may occur (Findlay 1938). Congenital transmission of maternal antibodies has been noted, though the virus itself does not appear to pass through the placenta (Levine *et al.* 1943). A few laboratory infections have been reported (Harrop *et al.* 1941). In adults infection may remain latent for months or years. Pathologically, the essential lesion produced by the virus is a low-grade inflammation of the lymph spaces and lymph channels leading to caseation and fibrosis.

Intracerebral inoculation of infective material into monkeys gives rise, after an incubation period of 6–12 days, to meningo-encephalitis. The mouse is also susceptible. After intracerebral inoculation, symptoms appear in 2–4 days; the coat is roughened, the back is hunched, and purulent conjunctivitis may be seen; death occurs in 3–8 days. The virus may be carried on indefinitely by passage, but some strains gradually become inactive. According to Miyagawa and his colleagues (1936*a*), their virulence may be restored by intradermal passage through guinea-pigs, in which the virus produces a caseous papule at the site of inoculation. Intranasal inoculation of mice may give rise to focal lesions in the lungs (van den Ende and Lush 1943). Guinea-pigs and rabbits are relatively resistant to intracerebral infection. Infective monkey or mouse brain, when inoculated on to the prepuce, is able to reproduce the typical disease in human subjects (Levaditi *et al.* 1932, 1935, Wassén 1935).

Ætiology.—The causative virus passes through Berkefeld N and Chamberland L3 candles, though the results are said to be inconsistent. According to Miyagawa and his colleagues (1935*c*) and to Broom and Findlay (1936), its probable diameter is about 0·125–0·175 μ. It is destroyed by exposure to a temperature of 56° C. for 10 minutes, and is readily inactivated by glycerol and by 0·1 per cent.

formol. At 4° C. it remains virulent for about 3 weeks. The virus is difficult to grow in tissue culture (Miyagawa *et al.* 1936*b*), but it can be propagated indefinitely by passage through the yolk sac of the developing chick embryo (Rake *et al.* 1940, 1941). An infection occurs that proves fatal to the embryo in 3 days to 2 weeks. Virus is present in large quantity in the wall of the yolk sac and in the yolk itself. Elementary bodies, demonstrable by histological methods, have been described by various observers in infected tissue from man, monkeys, and mice (for references see Findlay *et al.* 1938). They are sometimes referred to as the granulo-corpuscles of Miyagawa (Miyagawa *et al.* 1935*a*, *b*). Neutralizing antibodies may be detected in the serum of patients suffering from the disease and in the serum of inoculated rabbits. According to Rake and Jones (1944) the virus is able to give rise, when grown in the yolk sac of the chick embryo, to a thermolabile endotoxin killing mice, injected intravenously, in 12 to 24 hours.

Observations by Findlay, Mackenzie and MacCallum (1938) have shown that in the brain of mice the elementary bodies, which stain reddish-purple with Giemsa, swell up to 3 or 4 times their original size, forming large bodies, which take on a bluish-purple tint. These are grouped in a compact morula-like mass, probably intracellular in situation. Later some of the bodies break away and enter other cells. Gradually the large forms disappear, and are replaced by masses of small forms, which may be collected within cyst-like spaces. From these, the small forms or elementary bodies are liberated, and are again capable of infecting fresh cells. This sequence of small and large forms has been described as a developmental cycle, but the picture is so similar to that which is seen in ordinary bacterial cultures (see Chapter 4) that it is probably wiser not to use the expression. For a pictorial representation of cytoplasmic inclusion bodies in the brain of the experimentally infected mouse, the coloured plates in a paper by Ishimitsu (1937) may be consulted as well as those of Findlay and his colleagues. Elementary bodies may likewise be demonstrated in yolk sac cultures (Rake *et al.* 1941).

Diagnosis.—In the diagnosis of the disease the Frei (1925) test is widely used. The antigen consists of pus from a bubo, heated to 60° C. for 2 hours on 1 day, and for 1 hour on the following day. Intracutaneous inoculation of 0·1 ml. gives rise in an infected subject to an infiltrated inflammatory area, at least 0·5 cm. across, with a small central zone of necrosis. The reaction reaches its height after about 48 hours. Injected intravenously, the antigen gives rise to a febrile response; according to Hellerström this method is more sensitive than the original intradermal test.

Owing to the difficulty of obtaining and of standardizing test material of human origin, an antigen for intradermal use has been prepared from mouse brain. It has been reported on favourably by Grace and Suskind (1936). More recently an antigen, known as *lygranum*, prepared from cultures in the developing chick embryo, has been introduced. It is said to give rise to fewer non-specific reactions than the mouse-brain antigen, though a control test with normal chick embryo material must always be carried out (Shaffer *et al.* 1942, Axelrod 1942, Heisel *et al.* 1943). In practice, 0·1 ml. is inoculated intradermally. Readings are made at 48 and 72 hours. A papule not less than 7×7 mm. is regarded as a positive reaction, one less than 5×5 mm. as a negative reaction. The same antigen may be used for a complement-fixation test with the patient's serum. In view of the antigenic affinity of some of the pneumonia viruses (see p. 1875) with the virus of lymphogranuloma, the strict specificity of the skin and complement-fixation reactions must be regarded for the present as open to question (see Blair 1944, Beeson and Miller 1944).

Animal experiments suggest that the disease is susceptible to treatment with the sulphonamides. How these drugs act is unknown. They have no apparent effect on the virus *in vitro* (MacCallum and Findlay 1938), nor can any serious destructive action be demonstrated in the brain of mice, even though mice suffering from meningo-encephalitis are said to be cured by the administration of sulphanilamide or sulphaguanidine (Rodaniche 1942). Clinical experience of these drugs in the treatment of the disease in man has so far been unsatisfactory (Kramer *et al.* 1944).

PSITTACOSIS

This is primarily a disease of birds, which sometimes attacks man. The first human case reported was by Ritter (1879–80) in Switzerland, who described the clinical and pathological features of the disease. Little attention was paid to the disease till 1929–30 when psittacosis appeared in many places throughout the world. In the United States alone 74 foci of infection were recognized, and 169 cases occurred with 33 deaths (Armstrong 1930). The incubation period in man is usually about 6–15 days. The disease resembles typhoid fever in many respects and is often complicated by pneumonia. Infection occurs by the inhalation of infective material excreted by parrots, parrakeets, love-birds (budgerigars), canaries, or other birds. Case-to-case infection is rare. Workers in a laboratory where the virus is being handled are particularly prone to develop the disease (McCoy 1934).

For many years psittacosis was regarded as a disease affecting primarily birds of the parrot family; but in 1938, following an epidemic of pneumonia among the inhabitants of the Faroe Islands, widespread infection was demonstrated among the fulmar petrels (*Fulmarus glacialis*), which breed on the coast and are used by the inhabitants as food (Haagen and Mauer 1938, Rasmussen-Ejde 1938, Bedson 1940). Infection of human beings was four times as common in women as in men, and was apparently contracted by the inhalation of infected dust during the plucking of the birds' feathers. Later, Meyer and Eddie (1942) in the United States found that chickens were infected; and Meyer, Eddie and Yanamura (1942) showed that latent infection was present in pigeons collected from different parts of the States. Indirect evidence of infection of turkeys and ducks has been brought by Eddie and Francis (1942). In this country the isolation of the virus from pigeons has been reported by Andrewes and Mills (1943). It is thus evident that psittacosis is a common avian infection. Usually it appears to remain latent, but under certain conditions it may give rise to clinical disease and prove fatal. Man is infected mainly from parrots and budgerigars, owing possibly to his closeness of contact with these birds.

In parrots the disease is characterized by diarrhœa, shivering, weakness, and apathy. Respiratory disturbances may develop, accompanied by croaking noises and the secretion of mucus. Recovery often occurs, and is followed by a considerable degree of immunity to fresh infection. Latent infection appears to be widespread among parrots, cockatiels, lorikeets, budgerigars, and similar birds in their native countries, while in many aviaries in Europe and America the disease is endemic (Meyer and Eddie 1934, Burnet 1935). Pathologically the disease in parrots is essentially an infection of the liver and spleen with occasional involvement of the lungs. Multiple necrotic areas are found in the liver, and the infective agent is present in the blood, internal organs, nasal discharges, and fæces (Rivers

et al. 1931). Infection can be transmitted experimentally to budgerigars, parrots, mice, rabbits, guinea-pigs, and monkeys by suitable methods of inoculation. In mice continuous passage of the virus is readily accomplished by intraperitoneal inoculation of infected liver and spleen suspensions. The animals become ill and die in 4 to 20 days; pneumonia is uncommon, but characteristic changes occur in the liver and spleen, similar to those in parrots. Injected intradermally into the shaved skin of the rabbit or guinea-pig, the virus gives rise to a local papular eruption similar to that caused by the virus of herpes. Intracerebral injection into monkeys, rabbits, and guinea-pigs is followed by a meningo-encephalitis, but intranasal or intratracheal instillation produces in monkeys a pneumonic disease similar to that seen in man (see Bedson and Western 1930, Gordon 1930, Rivers and Berry 1931). For a description of the pathological lesions in man and animals, see Lillie (1933).

Ætiology.—The disease was first thought to be due to Nocard's bacillus—an organism apparently identical with *Salm. typhi-murium*—but later work showed



FIG. 298.

Intracellular colonies of psittacosis virus in rabbit cornea. From a tissue culture. On the right the colonies are situated outside the nucleus, which contains three nucleoli. On the left the colony is lying over the nucleus, but is not inside it. (× 1500.)

(From photograph kindly supplied by Dr. Robinow.)

that it was caused by a virus or a virus-like body. Bedson and his colleagues have been largely responsible for the ætiological investigation of the disease (Bedson *et al.* 1930, Bedson and Western 1930, Bedson 1932, 1936, Bedson and Bland 1932, 1934). They established the infectivity of human blood; they transmitted the infection to budgerigars and mice; they demonstrated the presence of virus-like bodies in infected tissues; they grew these bodies in tissue culture; and they showed that they were specifically agglutinated by psittacosis antiserum, and that they contained two different antigens, one heat-labile and one heat-stable, both of which were demonstrable by the complement-fixation reaction. Microscopical examination of suitable infective material, such as the tissues of mice suffering from the disease, reveals the presence of minute coccal and bacillary bodies, arranged singly and in pairs, and bearing a resemblance to rickettsiæ and to Paschen granules. They were described almost simultaneously by Levinthal (1930) in Germany, Coles (1930) in England, and Lillie (1930) in the United States, and are therefore sometimes referred to as L.C.L. bodies. They can be stained

with Giemsa, and better still with River's modification of Castaneda's rickettsial stain. They can be thrown down almost completely by centrifugation at 5,000 r.p.m. for 2 hours. They are largely held back by a Berkefeld N, a Chamberland L2, or a Seitz EK filter, but go through a gradocol membrane having an average pore diameter of 0·6 μ. Their probable size is about 200–300 mμ (Levinthal 1935, Lazarus *et al.* 1937). In tissue culture they increase rapidly in size, become embedded in a homogeneous ground substance, and are later replaced by smaller bodies (see Bland and Canti 1935). They can be cultivated on the chorio-allantoic membrane of the developing chick embryo for months without loss of virulence for mice, and can be consistently recovered from the spleen and liver of the embryo, which usually dies in 3–4 days (Burnet and Rountree 1935, Lazarus and Meyer 1939). They cannot be grown in the absence of living cells, nor do they appear able to grow extracellularly in tissue cultures (MacCallum 1936). They survive in frozen mouse spleen for 2 months, but die out rapidly in 50 per cent. glycerol phosphate at 6° C. Injected into guinea-pigs, they give rise to the production of agglutinins. The nature of these bodies is still in doubt, but in their general characteristics they would appear, as already pointed out, to be intermediate between the rickettsiæ and the filtrable viruses.

Owing to minor differences in pathogenicity of virus strains isolated from different avian hosts, Meyer, Eddie and Yanamura (1942) have suggested that the term psittacosis should be reserved for human infections contracted from psittacine birds, and the term ornithosis used for infections from petrels, pigeons and chickens. The wisdom of such a suggestion is open to doubt. If the practice should become established of coining a special name for the disease caused by every antigenic or pathogenic variant of an organism, then the whole nomenclature of the bacterial and virus infections will have to be revised.

Diagnosis and Immunity.—A rapid diagnostic test for the human disease, consisting of intraperitoneal injection of the patient's sputum into mice and the subsequent demonstration of elementary bodies in impression smears from the liver and spleen, has been described by Rivers and Berry (1935). The virus may be present in the patient's blood during the first four days of illness. The serum of convalescent patients contains complement-fixing bodies, but has little neutralizing power (Bedson 1933). Post-mortem material should consist of portions of pneumonic lung, any pleural or pericardial effusion, the liver, and the spleen. Suspensions, lightly centrifuged or passed through a coarse filter, should be injected intraperitoneally into mice; negative results, however, are common. The demonstration of virus in suspected birds is best accomplished by direct microscopical examination and by mouse inoculation (see Report 1937).

Vaccination experiments are still in the early stage. Bedson (1933, 1938) has been able to immunize mice by inoculation with a 0·1 per cent. formolized vaccine, in which no living virus could be demonstrated. Protection is not complete, even against small doses; infection almost invariably results, but remains latent—often for several months. Specific neutralizing bodies appear in very low titre in the blood of the vaccinated animals (see also Rivers and Schwentker 1934, Yanamura and Meyer 1942). According to Heilman and Herrell (1944), penicillin is of value in treatment of the experimental disease in mice, but it is doubtful whether it is similarly effective in human beings. For a general description of the disease and investigations on it, see also Sturdee and Scott (1930), Branham *et al.* (1930), Rivers (1934), Lazarus and Meyer (1939), Enders (1914).

Pacheco and Bier (1930), it may be noted (for further references, see Rivers and Schwentker 1932), described a disease of parrots in Brazil which closely resembled psittacosis in its clinical picture and in the macroscopic focal areas of necrosis found in the liver at post mortem, but which differed from psittacosis in certain respects. Thus, infection was confined to birds of the parrot family; it could not be transmitted to other laboratory animals; man appeared to be insusceptible; the virus passed readily through Berkefeld N and Chamberland B candles; and the parasitized cells contained acidophilic intranuclear inclusions. These observations were confirmed by Rivers and Schwentker (1932), who regarded the disease as undoubtedly different from psittacosis.

Smadel, Jackson and Harman (1945) have reported the isolation of a new virus from army pigeons suffering from an epizootic disease. It was usually associated with the psittacosis virus, but was occasionally found alone. It differed from the psittacosis virus in its smaller size, its more rapid growth on the chorio-allantoic membrane, its greater resistance to storage at — 20° C., its ability to give rise in the parenchymatous tissues of the liver to intranuclear inclusion bodies of the herpetic type, and its lack of pathogenicity for rabbits, guinea-pigs or mice. It is referred to as the I.N.I. (intranuclear inclusion) agent. Whether it is related to the virus producing intranuclear inclusions in parrots is doubtful.

VIRUS PNEUMONIAS OF MAN AND ANIMALS

A study of primary pneumonia during the past few years has revealed a considerable, and apparently increasing, proportion of cases that cannot be ascribed to infection with known bacteria. The majority of these cases have been characterized by a relatively mild illness of insidious origin, widespread lesions in the lungs as revealed by X-ray examination, a fairly prolonged course, absence of leucocytosis, insusceptibility to sulphonamide treatment, and a very low case mortality (see Francis 1944). Epidemiological studies by Dingle and his colleagues (1943, 1944) at Camp Claiborne, Louisiana, suggest that the disease has a fairly long incubation period—2 to 3 weeks—that it does not spread readily by contact, that it has no marked seasonal incidence, and that in all probability the pneumonic lesions represent merely a complication of a widespread respiratory infection, in much the same way as meningitis is sometimes superimposed on a basic epidemic of meningococcal rhinopharyngitis. On the other hand, groups of cases have been described by Reimann (1938) and others in which the disease has been very much more severe.

The ætiology of these pneumonias is still in doubt. Besides the rickettsiæ of Q fever (see p. 1850) several well-known viruses, such as the influenza virus, the psittacosis virus, the lymphocytic choriomeningitis virus, and the measles virus may give rise to pneumonia, but in many cases of the type of pneumonia we are now considering none of these viruses has been demonstrated. Numerous workers, however, have reported the isolation of viruses of one sort or another from human pneumonia patients; but it is as yet too early to form any clear picture of the relation of these viruses to each other or to the part they play in the causation of pneumonia in man and animals. Investigators have suffered from the necessity of using animals for purposes of isolation, rendering it impossible to tell whether the virus so obtained was derived from the patient or from a latently infected animal whose infection had been stimulated to activity by the introduction of

foreign non-specific material. It seems probable that more than one type of virus is concerned in the production of pneumonia, and that more than one type of clinical disease in man will eventually be defined. On this assumption we shall refer to this group of diseases as the " virus pneumonias," in preference to the common term " atypical pneumonias," which is either meaningless or misleading. Brief mention may be made of some of the more important viruses that have been isolated.

The *meningo-pneumonitis* virus was isolated repeatedly by Francis and Magill (1938) from ferrets that had been inoculated with throat washings of human patients suffering from an epidemic influenza-like disease. Whether it was derived from the patients or from the ferrets it was impossible to say. The virus passed through a Berkefeld V but not an N candle, and through a gradocol membrane of 430 $m\mu$ A.P.D., but not through one of 250 $m\mu$. It grew readily on the chorio-allantoic membrane of the developing chick embryo, but not in chick-embryo Tyrode tissue cultures. It was pathogenic for ferrets and mice by intranasal, subcutaneous, and intraperitoneal inoculation, and for monkeys, rabbits, and guinea-pigs by subcutaneous or intraperitoneal, but not by intranasal, inoculation. Intranasal inoculation of ferrets and mice was followed by pulmonary consolidation, from which the ferrets recovered and the mice usually died. Intracerebral inoculation of mice caused paralysis and death in 2 to 10 days, according to the dose.

Weir and Horsfall (1940) isolated a virus from pneumonia patients by intranasal inoculation of throat washings into the *wild mongoose* (*Herpestes griseus*). The virus passed through a Berkefeld N candle; it grew on the chorio-allantoic membrane of the developing chick embryo; it gave rise to pulmonary consolidation in the mongoose; it passed from mongoose to mongoose by contact; it was non-pathogenic for ordinary laboratory animals, including the ferret; and it was neutralized by sera of human patients convalescent from pneumonia.

Horsfall and Hahn (1940) demonstrated the presence of a virus—known as the *mouse pneumonia virus*—in normal mice. Under ordinary conditions it appeared to be avirulent, but by serial passage through mice, inoculated intranasally with infective lung, it became capable of causing a fatal disease characterized by pulmonary consolidation. The virus was about 100–150 $m\mu$ in diameter; it could be grown in chick-embryo Tyrode tissue culture medium; it was non-pathogenic to other laboratory animals; it was pathogenic to mice only after intranasal inoculation; and it was neutralized by about 30 per cent. of normal human sera.

Eaton, Beck and Pearson (1941) isolated a virus from four cases of pneumonia. It proved virulent for mice by intranasal or intracerebral inoculation, but not by intraperitoneal inoculation. It did not pass through a Berkefeld V candle, and it appeared to be closely related to the meningo-pneumonitis virus, though not apparently identical with it antigenically (Beck and Eaton 1942). Elementary bodies were demonstrable in impression smears from infected organs.

Gönnert (1941) described a virus found in the lungs of normal mice. It proved highly virulent after intranasal mouse passage, giving rise to broncho-pneumonia. In the tissues inclusion bodies were numerous. Elementary bodies were visible in large numbers, particularly in the leucocytes. The virus, which is referred to as the *broncho-pneumonia virus*, apparently passed through coarse filters, and bore a close resemblance to the viruses of lymphogranuloma, psittacosis and trachoma.

Nigg and Eaton (1944) described a pneumotropic virus met with in normal mice. It proved highly virulent on intranasal inoculation, giving rise in small doses to focal lesions in the lungs, and in large doses to extensive consolidation. In the earliest stages of infection elementary bodies could be demonstrated in the large mononuclear cells. The virus had no affinity for the nervous system. It could be cultivated in the yolk sac of the developing chick embryo. It was pathogenic to mice and hamsters, but not to rabbits. By complement-fixation and cross-immunity tests it could be distinguished from other

mouse pneumonia and meningo-pneumonitis viruses. According to Rake Shaffer, and Thygeson (1942) it is susceptible to sulphonamides.

Eaton, Meiklejohn, and van Herick (1944) were able to demonstrate a virus, derived from human pneumonia patients, by intranasal inoculation of cotton rats (*Sigmodon hispidus*). Pulmonary consolidation was produced, and the virus could be carried over by serial passage. It could be adapted to grow in the developing chick embryo. No elementary bodies were found in infected tissue, and the virus proved non-pathogenic to other laboratory animals. The virus was neutralized by the sera of human convalescents. Owing to the absence of demonstrable elementary bodies in the tissues, it is doubtful whether this virus should be included in the lymphogranuloma-psittacosis group.

Other viruses from human pneumonia patients have been described by Stokes, Kenney and Shaw (1939), Rose and Molloy (1943) and Karr (1943).

The relation of these viruses to each other is still under discussion. Complement-fixation, cross-immunity, and animal pathogenicity tests suggest that some of them, at least, are closely allied to the lymphogranuloma and the psittacosis viruses, and that with these they form a group analogous to that of the animal-pox or the pleuropneumonia viruses (see Rake, Eaton and Shaffer 1941, Eaton, Martin and Beck 1942, Pinkerton and Moragues 1942, Horsfall *et al.* 1943).

In some cases of virus pneumonia, "*cold*" *agglutinins*, i.e. agglutinins acting on human red corpuscles at 0° C. but not at 37° C., can be demonstrated in fairly high titre and may be of value in diagnosis (Turner 1943, Turner *et al.* 1943, Peterson *et al.* 1943, Horstmann and Tatlock 1943); though it must be remembered that they may occur in other diseases such as paroxysmal hæmoglobinuria, tropical eosinophilia and trypanosomiasis (see Viswanathan and Natarajan 1945). For references to papers on virus pneumonia in man, the reader should consult an article by Dingle and Finland (1942).

Cat Distemper

A disease, known variously as distemper, nasal catarrh, or influenza, has been studied to a limited extent in the cat (Hindle and Findlay 1933, Dalling 1932, 1934, Baker 1942, 1944). It appears to be caused by a virus entirely different from that infecting dogs. The disease is highly infectious, debilitating, and lasts about a month. It is characterized by sneezing and coughing, and a mucopurulent discharge from the eyes and nose. Pneumonia is not usually detectable during life, but if the animal is killed, greyish densely consolidated areas are found in the anterior lobes of the lungs. The disease can be reproduced in cats by intranasal infection with ground-up pneumonic lung; parenteral inoculation causes only a mild fever. Mice injected intranasally die in 3-5 days, and after serial passage of virus in 2–3 days. Infection can be transmitted to rabbits, guinea-pigs, and hamsters as well by intranasal inoculation, but only mice and hamsters die. Films from the lungs of these animals show dense plaques and aggregations of elementary bodies similar to those seen in psittacosis. The virus appears to be fairly large, as it does not pass Berkefeld N candles, except in very small amount. It can be grown in the yolk sac of the developing chick embryo. It is killed at 50° C. in 30 minutes. In 50 per cent. glycerol it is inactivated within about a month. The serum of convalescent cats contains specific complement-fixing bodies.

Baker's virus is similar in many respects to the virus of mouse pneumonia described by Nigg (1942). Thomas and Kolb (1943), who have compared the

two, find that they are related antigenically, not only to each other, but to the virus of psittacosis. According to Hamre and Rake (1944), Baker's feline pneumonitis virus differs from the meningo-pneumonitis virus (see p. 1876) in the nature of its toxin and corresponding antitoxin, and in the larger dose required to set up infection in mice by intracerebral inoculation.

Another virus isolated from feline pneumonia, but differing from Baker's virus, was described by Blake, Howard and Tatlock (1942). It may be noted that cases of pneumonia in human patients and in cats have not uncommonly been associated, though there is yet no definite evidence to show that the same virus was responsible for both.

TRACHOMA

Since Noguchi (1927, 1928) described the isolation of a very small Gram-negative bacillus, *Bact. granulosis* (see p. 901) from 4 out of 5 American Indians suffering from trachoma, extensive investigations on the ætiological significance of this organism have been carried out in different parts of the world, and a voluminous literature has sprung up.

Noguchi found that the inoculation of pure cultures of this bacillus beneath the conjunctiva and on to the scarified conjunctiva of *rhesus* monkeys was followed in 2 to 4 weeks by a granular conjunctivitis, which slowly progressed till after 2 to 5 months the inoculated conjunctiva closely resembled the human trachomatous conjunctiva in the early stages of the disease. The lesions were follicular; they extended over the tarsus, which became thickened; and in one monkey definite scar formation occurred. Histologically the lesions were closely similar to those found in human trachoma. The disease could be passed in series from monkey to monkey, and the organism could be recovered from the experimental lesions produced. The organism had no effect when inoculated into the conjunctiva of the rabbit.

Attempts to confirm Noguchi's findings have met with a varied degree of success. Olitsky (1930) and Olitsky, Knutti and Tyler (1931*a*, *b*, *c*) claim to have succeeded in isolating *Bact. granulosis* from natural trachoma in human beings and from monkeys infected with human trachomatous material, and to have conveyed the disease to *rhesus* monkeys by conjunctival inoculation with pure cultures of this organism. On the other hand, several workers including Wilson (1931) in Egypt, Bengtson (1932) in the United States, Schuurman (1932) in Java, and Tang and Chou (1935) in China have failed to isolate this organism during the investigation of large numbers of cases.

Two factors contribute to this confusion. The first is the varying definition of trachoma adopted by different workers: the second is the spontaneous occurrence in monkeys of a follicular conjunctivitis simulating in some respects trachoma in human beings (see Wilson 1928, Olitsky and Tyler 1933, Olitsky, Syverton and Tyler 1933). Trachoma is a chronic disease progressing from early conjunctival inflammation to late hypertrophy and cicatricial changes. According to Bland (1944*a*), the four characteristic signs are: (1) inclusion bodies—described as " Lindner's initial bodies " and " von Prowazek-Halberstaedter inclusion bodies " (see Bengtson 1928)—in conjunctival epithelial scrapings; (2) follicular reaction in the tarsus as well as in the fornices; (3) invasion of the cornea by blood vessels; and (4) subsequent scarring of the conjunctiva. Bland has found that grivet monkeys (*Lasiopyga griseoviridis*: syn. *Cercopithecus æthiops*) and vervet monkeys

(*Lasiopyga pygerythra*: syn. *Cercopithecus pygerythra*), both of which suffer from spontaneous folliculosis, can be infected with trachoma—as shown by subsequent inoculation of human volunteers—but that the trachomatous reaction cannot be distinguished clinically or histologically from folliculosis. These animals are therefore useless for most experimental purposes. Baboons (*Papio hamadryas*) are likewise prone to develop spontaneous folliculosis (Bland 1944*b*). On the whole, Bland (1944*c*) seems to regard *Macacus rhesus* as the most reliable experimental animal, though only about 50 per cent. of the animals are susceptible to trachoma.

Some workers, notably Busacca (1933, 1937) and Cuénod and Nataf (1936, 1937, 1938), regard the disease as a rickettsial infection.

Microscopical examination reveals the presence of tiny coccoid bodies, 0·2–0·3 μ in diameter, staining pale blue with May-Grünwald-Giemsa, occurring sometimes in enormous numbers in epithelial cells removed by gentle scraping from the conjunctiva. The name *Rickettsia trachomatis* has been suggested for them (Foley and Parrot 1937). The organism is said to have been cultivated in the louse's intestine and then to have reproduced the disease in monkeys and in a blind human eye. In addition numerous workers have reported a positive Weil-Felix reaction in trachoma, particularly in the acute stages. None of this evidence is entirely convincing. The occurrence of epithelial cell inclusions is equally compatible with a virus origin. The cultivation of rickettsiæ in the louse's intestine has not been confirmed by workers like Weigl (1939) and Trapesontzewa (1939), who took the precaution to work with lice free from *Rickettsia rocha-limæ*. Durand and Lumbroso (1938) in Tunis found, in contradistinction to Poleff and Nain (1938*a*, *b*) in Morocco, that control patients gave a Weil-Felix reaction almost as often as trachomatous patients. Moreover Kirk, McKelvie and Drysdale (1939), working in the Northern Sudan where typhus is absent, examined 200 sera from patients in different stages of trachoma, but failed to find a single one that agglutinated *Proteus OX* 2 or *Proteus OX* 19 above 1/25.

More recently, the conviction has been growing that the disease is of virus origin.

Julianelle, Harrison and Morris (1937) were able to confirm a previous observation of Nicolle and Cuénod (1921), namely that the infective agent remains alive for several days in the rabbit's testicle—an observation that may be utilized for freeing trachomatous material from contaminating bacteria. It does not, however, survive testicular passage. Filtration experiments were unsatisfactory; in nine attempts to filter the infective agent through a Berkefeld V candle or a collodion membrane of 0·6 μ A.P.D. the results were uniformly negative. Tissue cultures were likewise unsuccessful. The agent was found to survive in the ice-chest for 2 or 3 days as a rule, but exposure to a temperature of 45°–50° C. or to 25 per cent. bile at 37° C. inactivated it in 15 minutes. Thygeson (1934*a*) claims to have demonstrated the presence of elementary bodies, about 0·25 μ in diameter, in trachoma, and to have filtered them successfully through a collodion filter of 0·6–0·75 μ A.P.D. Rake, Shaffer and Thygeson (1942) have shown that the serum of trachomatous patients can fix complement in the presence of a lymphogranuloma antigen, suggesting that the infective agent of trachoma is related to that of lymphogranuloma inguinale. Likewise both organisms are reported to be susceptible to sulphonamides.

Julianelle (1938), who has reviewed the whole evidence in a monograph that should be consulted by those desirous of further information, sums up the known properties of the infective agent of trachoma as follows: low infectivity, even for man; occasional filtrability; high degree of tissue specialization; poor antigenicity; inability to grow in tissue culture; sensitivity to deleterious agents; and power of production of inclusion bodies in the invaded tissues. All these

properties suggest that the causative agent is a virus. The final proof of this conclusion is still awaited.

Though trachoma is said to be favourably influenced by treatment with the sulphonamides, it is very doubtful whether these drugs have any direct action on the virus. Such improvement as is observed seems to be more probably due to their influence on secondarily infecting agents (see Lavery 1945). It may be added that Bernasconi (1941, 1943) brings evidence to suggest that nutritional deficiency plays an important aetiological rôle and that the disease can be cured by the addition of Vitamins A and D to the diet.

Inclusion Blennorrhœa

Inclusion blennorrhœa is a disease that attacks the eyes of new-born infants and of adults. Though it closely resembles trachoma in many respects, the balance of evidence seems to be against the identity of the two diseases. The name inclusion blennorrhœa was coined by Linder (1911), who distinguished the disease in the new-born from gonorrhœal ophthalmia. According to Thygeson (1934*b*) and Thygeson and Mengert (1936), whose papers the reader should consult for references to the observations of previous workers, the onset of the disease in infants is 5 to 9 days after birth. It is characterized by the presence in some of the conjunctival epithelial cells of cytoplasmic inclusion bodies indistinguishable from those of trachoma, by its acute onset, its failure to produce destructive lesions of the cornea, its long duration—3 to 12 months—and its resistance to treatment. In adults the disease is sometimes referred to as *swimming bath conjunctivitis*, though it is clear that such a term includes common infections due to bacteria such as the Koch-Weeks bacillus as well as the disease we are now considering (see Julianelle 1938). The observations of Thygeson and of Julianelle, Harrison and Lange (1938) show that inclusion blennorrhœa can be transferred to monkeys, especially baboons, and that, in contrast to experimental trachoma, inclusion bodies can be demonstrated in the conjunctiva of the inoculated animals. Elementary bodies about 0·25 μ in diameter are found in infective material; they can just pass a collodion filter of 0·7 μ A.P.D. Tissue cultures have so far been unsuccessful. The disease does not seem to be highly contagious. There is evidence that it affects primarily the genital tract, and that the eye is infected by contamination with material from this source. Like trachoma, it is said to be susceptible to sulphonamides (Rake *et al.* 1942), but this is doubtful.

It may be mentioned that the disease *Heartwater of cattle* is sometimes included in this group, but as the evidence is not entirely conclusive, we have considered it best to leave it in the rickettsial diseases (see p. 1862).

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CHAPTER 86

FILTRABLE VIRUS DISEASES—*Continued*

B. GROUP CHARACTERIZED BY LESIONS OF THE SKIN

VARIOLA AND VACCINIA

Variola, or smallpox, is a disease of great antiquity, of widespread distribution, affecting both sexes at all ages, and occurring both endemically and epidemically. According to Rajchman (1928) there were over 300,000 cases of the disease in the world in 1927; in India alone it causes about 50,000 deaths annually. In recent years, however, smallpox has been characterized by an almost universal decrease in the case-mortality rate. There seems little doubt that this is due largely to the occurrence in many localities of a mild type of disease, usually referred to as "variola minor" or "alastrim." There are in fact two entirely distinct types of the disease: (1) the classical type, variola major, with severe symptoms and a case-mortality rate of 10–30 per cent.: (2) the mild type, variola minor or alastrim, with mild symptoms and a case-mortality rate of only 0·1–0·3 per cent. In some countries the two types may coexist side by side; thus in 1922 there was an epidemic of the mild type in the Midlands and North of England, in which 895 cases were reported with only 3 deaths—a case-mortality rate of 0·3 per cent.—and a smaller epidemic of the classical type in London, in which 78 cases were reported with 24 deaths—a case mortality of 31 per cent. (see also Report 1938). In the United States the number of reported cases fell from 42,679 in 1930 to 4,346 in 1934—apparently as the result either of the replacement of variola major by alastrim, or of the widespread immunization of the population against variola major by unrecognized infections with alastrim (see Russell 1941). Whether alastrim is, as the name variola minor implies, merely a mild form of smallpox or a separate disease is still in doubt (see p. 1889).

The mode of infection in smallpox is not clear. It used to be thought that infection occurred from the inoculation of the skin with dried pustules and scabs, but this is now believed to be an unusual method. Epidemiological and experimental evidence points to infection by the nasopharyngeal route. The observations which indicate that infection is not usually carried by dried pustules and scabs are: (1) cases of smallpox occur without a rash which nevertheless prove infectious; (2) experimental inoculation of the skin with variola seldom leads to more than a mild disease with imperfectly developed pocks. On the other hand, the virus is said to be generally present in the nasopharynx of patients, and to be sometimes demonstrable in this situation even during the incubation period. Von Wasielewski and Winkler (1925), who review the evidence, conclude that the virus is coughed out in droplets, and gives rise to infection of the mucosa of the upper respiratory

tract; from the infected mucosa the virus is then distributed to all parts of the body.

That variola is primarily caused by a filtrable virus, there is no doubt. Filtration experiments are not, however, always successful, probably because of the imperfect liberation of the virus from the cells, or its adsorption by particulate matter in the suspension (see Green and Eagles 1931). During the disease the virus appears to be widely distributed throughout the body; it has been demonstrated in the blood stream (Ohtawara 1922, Parker and Nye 1925*a*, von Wasielewski and Winkler 1925, Haagen 1928). Though Levaditi (1924) maintains that the chief manifestations of the disease result from an affinity of the virus for the skin, there is now little doubt that the primary action of the virus is on the mesoderm; for this reason it is sometimes referred to as "viscerotropic" (see Ledingham 1924, Hurst 1936).

The cultivation of the virus of variola, and of the closely associated vaccinia virus, in the rabbit's testicle, in tissue cultures *in vitro*, and on the chorioallantoic membrane of the developing chick embryo, has already been described (Chapter 41).

Animal Inoculation.—Smallpox cannot be reproduced in its typical form in any of the lower animals. In calves and rabbits only minimal lesions are produced; and if material from these lesions is passed continuously through a series of animals, the virus is so altered that it gives rise to vaccinia or cow-pox, and reproduces this type of infection when again inoculated into a child.

The most susceptible animal to *variola* is the *monkey*. Intracutaneous inoculation of pus from a human patient gives rise to a papule after 5 days; by the 8th day this is vesicular, and by the 10th day a typical pock is formed, with a raised congested edge surrounding a crateriform depression, partly filled with dark purulent material (Blaxall 1923, Gordon 1925). Intratesticular inoculation of the monkey with variola virus gives rise to an orchitis with the appearance of a rash on the scrotum. Passage from one monkey to another is almost uniformly successful. Cats and dogs are said to be less susceptible than monkeys, but more so than rabbits. Infection in the less susceptible animals may be of the "inapparent" type, in which immunity develops in the absence of any clinical manifestations of disease (Teissier *et al.* 1931). Pus from cases of alastrim gives rise to very much the same lesions as that from the classical type of smallpox.

Both calves and rabbits can be readily infected with *vaccinia*. Inoculation of the scarified skin of a *rabbit* with calf lymph causes a red swelling on the 3rd day, which increases until a red papule is formed, surrounded by a red areola; the papules become vesiculated about the 5th day, and tend to coalesce and to become pustular; scabbing follows on the 7th or 8th day. A good calf lymph should give a reaction in the rabbit when diluted 1/1000; sometimes a reaction occurs even in a 1/100,000 dilution. If vaccine lymph is inoculated intravenously into a rabbit, and a portion of the skin is thereafter shaved, vaccinial papules appear about the 3rd day on the shaved area (Calmette and Guérin 1901). In poorly nourished and in nursling rabbits the susceptibility of the skin to infection is said to be decreased (Sprunt 1942, Pearce *et al.* 1936). If a very large dose of vaccine lymph is inoculated intravenously into a rabbit—1 to 2 ml. of undiluted stock emulsion—or a highly virulent virus is employed, a generalized eruption occurs over the entire body surface, and lesions may develop in the internal organs (Noguchi 1918, Douglas *et al.* 1929, Armstrong and Lillie 1929). Subcutaneous inoculation may give rise to a local pustular eruption, but often no superficial lesion appears at all. Intratesticular inoculation results in the development of an orchitis and œdema of the scrotum; the animal dies about the 6th day. Inoculation on the scarified cornea gives rise to a keratitis and purulent conjunctivitis. Intracerebral inoculation of rabbits is followed

by trembling, paralytic, and other nervous symptoms and by death on the 5th to the 8th day; the brain and spinal cord of these animals prove infective to fresh rabbits, when inoculated on to the cornea or intracerebrally (Marie 1920). After intranasal inoculation, the virus can be demonstrated in the cervical lymph glands in about 12 hours; it appears to become attached to the lymphocytes and hence finds its way into the blood stream (Yoffey and Sullivan 1939).

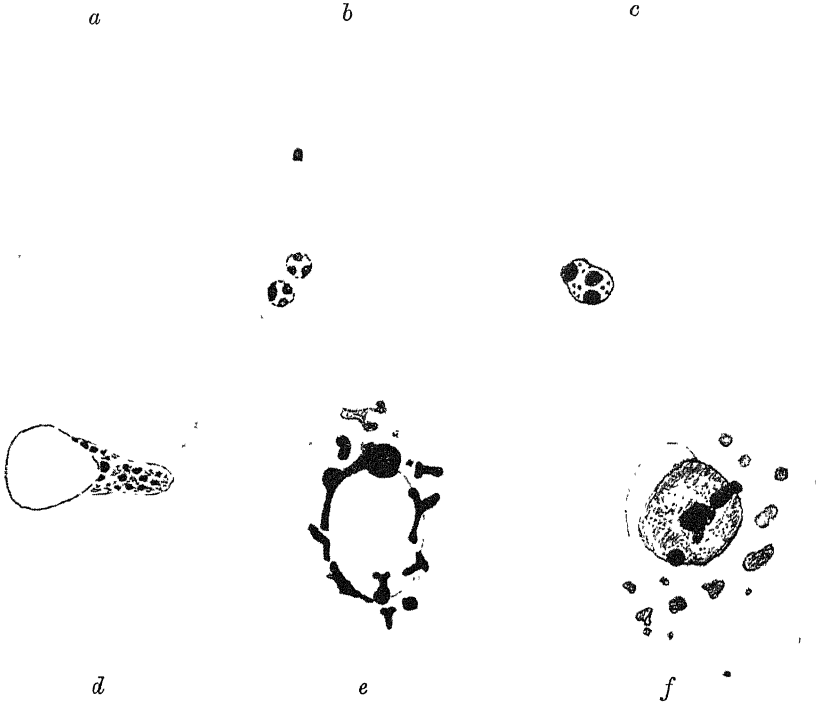


FIG. 299.

Guarnieri corpuscles in corneal cells of rabbit experimentally inoculated with vaccinia virus.

a = normal corneal cell.
b = cell one day after inoculation, containing small vaccine bodies.
c = cell 2 days after inoculation, containing a solitary vaccine body in which large and small droplets may be distinguished.
d = cell 4 days after inoculation, showing a vaccine body partly surrounding the nucleus.
e = cell 5 days after inoculation, showing vaccine bodies surrounding the nucleus.
f = cell 5 days after inoculation, containing numerous vaccine bodies.

(After Cowdry 1927.)

In *mice* a vesiculo-pustular reaction may often be obtained by inoculation of the scarified foot pad or tail with vaccinia virus (Rosenau and Andervont 1931).

Histologically, the main lesion in the rabbit inoculated intracutaneously is a diffuse perivascular infiltration of the dermis; the changes in the epidermis appear to be secondary to this. According to Ledingham (1924) the vaccinial lesion is essentially an acute infective granuloma in which the reticulo-endothelial system is primarily and dominantly involved. By continued passage through the brain of rabbits, the virus may acquire more or less fixed properties—like the virus of rabies—causing death regularly in 5 to 8 days (Marie 1920). To this fixed virus the term "neurovaccine" is applied

by Levaditi and Nicolau (1923). The virus, though having a special affinity for the nervous tissue, is still able to produce typical lesions on cutaneous inoculation.

Guarnieri Corpuscles and Elementary Bodies.—As long ago as 1892 Guarnieri (1894) observed in the lesions of variola and of vaccinia peculiar intracellular bodies, which he regarded as being of protozoal nature. When stained by methylene blue, the body shows a slightly stained spherical mass lying eccentrically, and surrounded by a clear area, which in its turn is encircled by a number of small granules. These Guarnieri corpuscles may be produced very rapidly—in a few hours—by inoculation of the scarified cornea of a rabbit with the vaccinia virus. Fixed, and stained with methylene blue and eosin, they appear as irregular pink and lilac masses, which indent the nuclei, and which may contain acidophilic or basophilic granules (Figs. 299, 300). Besides the Guarnieri corpuscles, which are always present at some stage in variolous and vaccinial lesions, very tiny intracellular bodies, 0·1–0·25 μ in diameter, have been described ; they are generally called "Paschen's granules" (see Fig. 232, p. 953). The work of Ledingham and his colleagues, of Paschen, and of others (see Chapter 41) leaves no doubt that the Paschen granules, or elementary bodies as they are often called, constitute the actual causative virus of the disease. The Guarnieri corpuscles probably represent intracellular microcolonies of the virus. The microscopical demonstration of the virus particles *in situ* may be accomplished by suitable staining methods (see Taniguchi *et al.* 1932*a*, Haagen and Kodama 1934, Herzberg 1934, 1936, Tang and Wei 1937). For a description of the general properties of vaccinial elementary bodies, the reader is referred to the review by Smadel and Hoagland (1942).

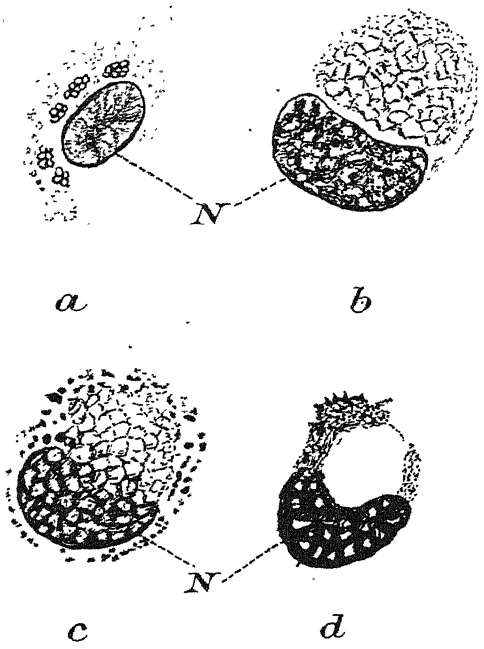


FIG. 300.

Guarnieri corpuscles in the cornea of an experimentally inoculated rat.

a = five small vaccine bodies in congeries.
b = fully developed vaccine body.
c = vaccine body showing reticulum breaking up into granules ; similar granules throughout cytoplasm.
d = vaccine body with central portion consisting of homogeneous material.
N = nucleus.

(After Ewing.)

Laboratory Diagnosis of Smallpox.—The antigenic structure of the vaccinia virus has already been considered in Chapter 41. The serological diagnosis of smallpox is made by mixing a suspension of ground-up variolous material with

an antivaccinial rabbit serum, and incubating at 37° C. A positive reaction is marked by the appearance of a finely floccular precipitate, similar to that obtained with vaccinial material (Gordon 1925). Using this method, Tulloch (1928) was able to make a rapid diagnosis of smallpox during the course of an epidemic in Dundee. Since then, the value of the flocculation test has become increasingly obvious. The careful studies of Burgess, Craigie and Tulloch (1929) and Craigie and Tulloch (1931) show that it constitutes an important practical method in the rapid diagnosis of doubtful cases of smallpox. For a description of the detailed technique reference should be made to the report by Craigie and Tulloch (1931). The serum of patients suffering from variola may also agglutinate suspensions of elementary bodies (Amies 1932). The reaction appears to be specific for variola and vaccinia. Some discussion has arisen over its real nature, but it is probably a mixed precipitation and agglutination reaction (see Craigie 1932, Gordon 1935*a*, *b*, Ledingham 1935*a*, and Chapter 41). Diagnosis may also be made by the complement-fixation test using a suspension of crusts or of vesicular fluid as antigen. According to Craigie and Wishart (1936) this test is 8–10 times as sensitive as the flocculation test. More recently van Rooyen and Illingworth (1944) have recommended the direct microscopical examination of suitably stained smear preparations from scrapings of papules or vesicles. The elementary bodies, of variola and vaccinia are said to be larger, more numerous, and to stain deeper than those of varicella.

Tests depending on animal inoculation are less satisfactory. McKinnon and Defries (1928) and Defries and McKinnon (1928) find that the intracutaneous inoculation of variolous material into rabbits produces a reaction characterized by a red palpable swelling, commencing on the 2nd day and reaching its maximum about the 4th day, when it appears as a firm, elevated plateau, 10–20 mm. in diameter; subsequently the central red area becomes straw-coloured, and desquamation or crust formation may occur; the lesion disappears by the 12th day, leaving no scar. On the other hand, if the variolous matter is injected into vaccinated rabbits, either no reaction occurs at all, or there is an allergic reaction, characterized by the rapid appearance and disappearance of a red tense nodule (Blaxall 1923). Material from varicella, as a rule, gives rise to no reaction in either normal or vaccinated rabbits, though on this point the evidence is conflicting (Hull and Nauss 1926). Paul's test, which consists in the production of a keratitis by inoculation of the rabbit's cornea with variolous material, is sometimes used in diagnosis, but according to Defries and McKinnon (1928) it is positive in only 50 per cent. of cases. By inoculating pustular fluid on to the chorio-allantoic membrane of the developing chick embryo, and examining for pocks after 2–4 days' incubation at 35°–36° C., a positive diagnosis may sometimes be made (Bohls and Irons 1942); but owing to non-specific reactions and other pitfalls this method should be used with caution.

Immunity.

Our whole knowledge of immunity may be said to have started with Jenner's discovery in 1796 that inoculation with cow-pox protects against subsequent inoculation with smallpox. He believed that cow-pox was really smallpox, modified by passage through the cow—a belief to which investigations of recent years have lent considerable support. Cutaneous inoculation of the calf with variolous material from man produces insignificant lesions; but if the material from these lesions is passed through further calves in series, then after three or more passages a good vesiculo-pustular eruption appears on the inoculated skin. This experimentally produced disease is apparently identical with the naturally occurring cow-pox. If the pustules are scraped at the height of their develop-

ment, ground up in a mortar, and mixed with glycerolated saline, the resulting product is indistinguishable from vaccine lymph. In practice it is found beneficial to pass the vaccinia virus from the calf to a rabbit, from the rabbit to a calf, from the calf to a child, and from the child back to a calf, or through some similar series; in this way the activity of the virus seems to be better maintained than by continuous passage through one species of host.

Experimentally, rabbits that have been inoculated with vaccinia virus, either cutaneously, subcutaneously, or intravenously, become resistant to a second inoculation within about 10 days. If repeated injections are given, the rabbits become hyperimmunized, and in their serum neutralizing viricidal antibodies can be demonstrated, as well as agglutinins, precipitins, and complement-fixing bodies (Ledingham 1924, Gordon 1925, Sobernheim 1925). W. Smith (1929) has brought evidence to show that, in rabbits inoculated intravenously, the virus is rapidly taken up by the formed elements of the blood—probably the leucocytes—and remains in the circulation for at least 8 days. (See also Douglas and Smith 1930, Gildemeister and Hilgers 1930.) In order to demonstrate its presence, it is necessary, however, to fractionate the blood, and use only the washed cells for injection. If the whole blood is injected, the presence of the virus is masked by the antibodies, which become demonstrable in the plasma about the second day after infection, and increase up to the 6th or 7th day. This method of demonstrating the virus in material which, when injected in the original state, proves to be non-infective, may have an important bearing on future investigations into the distribution of the filtrable viruses within the body. (See also p. 1233.)

By whatever route the virus is introduced, provided an active strain is used, a general immunity develops. There is a partial exception to this in the cornea; a vaccinial keratitis renders the affected cornea resistant to further inoculation, but not necessarily the opposite cornea or the skin; similarly cutaneous vaccination, though rendering the skin refractory, may yet leave the cornea susceptible (Levaditi and Nicolau 1923). According to Sobernheim (1925) this statement must be modified; he brings evidence to show that after cutaneous inoculation the cornea does become immune to vaccinia, but the immunity develops more slowly in the cornea than in the skin—probably because of its poor blood supply. There seems little doubt, therefore, that immunity to vaccinia is a general and not a mere local immunity, and is dependent, to some extent at least, on the development of humoral antibodies.

Monkeys inoculated with vaccinia are completely protected against subsequent inoculation of variola (Gordon 1925); monkeys inoculated with variola or alastrim are either partly or completely protected against subsequent inoculation with vaccinia (Blaxall 1923, Gordon 1925). Immunologically the alastrim virus resembles the variola virus very closely, but minor differences are apparent in the degree of protection afforded by the different viruses against vaccinia. Generally speaking, vaccinia affords almost perfect protection against variola and alastrim, but the protection afforded by variola or alastrim against vaccinia is not so complete. It must be mentioned, however, that there is a type of mild smallpox in Australia which affords practically no immunity either to variola or vaccinia, and to which vaccinia or variola affords only incomplete protection (Ledingham 1925). The identity of alastrim with mild smallpox is open to question. Though cross-immunity between the two diseases suggests that they are caused by essentially the same virus (see Leake and Force 1927, Diem 1930), epidemiological evidence

does not wholly support this view (see Findlay 1936). Moreover, according to Torres (1936), the histological changes in the two diseases are distinctive. Findlay (1936) believes that smallpox and alastrim are caused by separate viruses, each of which breeds true, and each of which can give rise to vaccinia virus.

Again, the relation of vaccinia to variola, and of vaccinia to cow-pox is still in doubt. Most authorities agree that the vaccinia virus is a modified form of the variola virus. Nevertheless, by agglutination it can be shown that, though the two viruses possess a common antigen, they are not identical (Ledingham 1935*b*). It has been generally assumed, since the days of Jenner, that vaccinia is variola modified by passing through the calf, and that vaccinia is essentially the same disease as naturally occurring cow-pox. Downie (1939*a*, *b*), however, has pointed out that vaccinia and cow-pox viruses differ in their heat-labile antigens, and that they give rise to different lesions in animals; he would therefore regard the two viruses as being distinct.

Experiments in rabbits indicate that the highest immunity to vaccinia is produced by living virus. Gordon (1925) found that heat diminished the antigenic activity of the virus very considerably. Thus, to produce the same degree of protection as that afforded by raw virus, about 20 times as much virus heated to 55° C. for 30 minutes had to be employed; lymph heated to 65° C. had only 1/100 of the immunizing power of the 55° C. lymph, while lymph heated to 100° or 120° C. had practically no immunizing effect at all. According to Nakagawa (1924), lymph heated to 100° C. for 40 minutes still has some immunizing power, but very large doses have to be employed to demonstrate it. Virus treated by exposure to 1 per cent. phenol for 24 hours at 37° C. had much the same immunizing power as the 55° C. lymph.

There is no question that the antigenic power of heated, phenolized, or formolized vaccinia virus is very much less than that of the untreated virus. Its power of stimulating antibody production, especially protective antibodies, seems to be very slight, though it can still serve as an efficient hapten in the demonstration of skin sensitivity in a sensitized human subject or animal (see Kaiser 1929, Zehnder 1929, Bussel and Maysner 1930, Andervont and Rosenau 1930, Kraus 1931, Hilgers 1931, Craigie and Wishart 1933, Parker and Rivers 1936, Bernkopf and Kligler 1937). After vaccination of rabbits with raw lymph, immunity may be detectable about the 4th day; during the next 2 days it increases rapidly, and by the 10th day a substantial immunity is attained.

Prophylaxis and Human Vaccination.—It is scarcely necessary to describe here the prophylactic measures that should be taken on the discovery of a case of smallpox in a country in which it is normally non-endemic. They comprise the removal of the patient to an isolation hospital, disinfection of the patient's house, including steam disinfection of all infected clothing and bedding, daily supervision for 16 days of all contacts, vaccination or revaccination of contacts, and if thought desirable of the general population, exclusion of contacts from schools, factories and other places of congregation, and careful observation of all doubtful cases of disease.

Inoculated cutaneously into a fully susceptible human subject, vaccinia virus gives rise, after an incubation period of 3–4 days, to a papule, which on the 6th or 7th day becomes vesicular. The vesicle, which is multilocular, umbilicated, and surrounded by a red areola, progresses to the pustular stage between the 10th and 12th days, after which retrogression sets in and the pustule dries up. The crust becomes detached about the 21st day, leaving a red pitted scar—the

so-called foveation—which in the course of years turns white. The size of the reaction is dependent on the concentration of virus in the lymph (von Gröer 1935). A rise in temperature may occur about the 4th day, reaching its maximum about the 8th day; there may also be some enlargement and tenderness of the regional lymph nodes. A second inoculation made some years later may be followed by much the same reaction, or it may be accelerated—*vaccinoid reaction*—the papule appearing earlier and completing its evolutionary course in a shorter time. In persons recently vaccinated the so-called *immune reaction* is common; this consists of a papule that reaches its maximum size in 8 to 72 hours, and disappears without passing through the vesicular or pustular stage. There is evidence that the rate at which the pustule develops is an index of immunity resulting from the previous vaccination (McKinnon and Defries 1931).

For human vaccination, calf lymph is generally employed, but it has been shown that lymph taken from vaccinial lesions in other animals, such as the sheep or the water buffalo, can be used satisfactorily. Of late years two other types of vaccine have been introduced, partly to provide a bacteria-free product, partly to avoid the severe local and constitutional reactions to which calf lymph sometimes gives rise, and partly in the hope of preparing a vaccine of constant virulence. The first is the so-called Rivers vaccine, prepared by growth of the virus in a chick embryo tissue culture medium (see Rivers 1931, Rivers and Ward 1935); the second is the vaccine produced by Goodpasture and Buddingh (1933, 1935), who cultivate the virus on the chorio-allantoic membrane of the developing chick embryo. Neither of these vaccines has yet been tried on a sufficient scale to justify a critical assessment of their value. The available evidence suggests that neither is quite as good as calf lymph in the degree of protection to which it gives rise, but further observations are desirable (see Buddingh 1937, 1943, Lehmann 1938, Stevenson and Butler 1939, Rivers, Ward and Baird 1939, Ellis and Boynton 1939, Donnally 1939).

Vaccination itself may be carried out by scarification, by the multiple pressure method (see Leake 1927), by intracutaneous injection (see Force 1927, Toomey and Hauver 1928, 1929), or by subcutaneous inoculation (see Yaoi 1934, 1936). None of these methods seems to be effective unless an epidermal vesicle is produced; and as the intracutaneous and the subcutaneous methods often fail to give rise to vesicle formation, their use is not to be strongly advocated (see Henderson and McClean 1939). The desire to reduce the local vaccinial lesion to a minimum has led many workers to advocate the use of vaccination by the multiple pressure method or by a single tiny scratch; but the evidence given below on the relation between the amount of foveation and the diminution in the case mortality from smallpox should suggest caution in the adoption of these methods. For primary vaccination during infancy or early childhood and for re-vaccination there is much to be said for the use of the 3- or 4-scratch method, each scratch being about $\frac{1}{4}$ inch long. The milder methods of vaccination are best reserved for late primary vaccinations and for persons in whom it would be unwise to risk a severe local or general reaction.

Protective Effect of Vaccination.—The evidence that vaccination protects against smallpox is very strong, but unfortunately exact statistical evidence is difficult to obtain. This holds particularly with regard to protection against infection as opposed to death; for, from the nature of the case, it is impossible in a diffuse population to be certain of the degree of risk to which the individual

members have been exposed. On the other hand, a large body of statistical evidence has been accumulated to show that the case mortality from smallpox is greatly diminished by vaccination. This evidence is as follows:

(1) Vaccination diminishes the case mortality. Table 183 gives the figures collected by Cameron (see Report 1928*a*) for the case mortality of smallpox in vaccinated and unvaccinated persons treated in the Metropolitan Asylums Board Hospitals during 1901–04.

TABLE 183

Year.	Vaccinated.			Unvaccinated or Doubtful.		
	Admitted.	Died.	Percentage Case Mortality.	Admitted.	Died.	Percentage Case Mortality.
1901 . . .	1,282	127	9·9	461	165	35·8
1902 . . .	5,663	578	10·2	2,253	759	33·6
1903 . . .	245	7	2·9	110	5	4·5
1904 . . .	289	14	4·8	160	13	8·1
Totals . .	7,479	726	9·7	2,984	942	31·57

It will be seen that the mortality amongst the unvaccinated was more than three times that amongst the vaccinated. Macdonell (1901–02) found that in the outbreaks in Sheffield, Dewsbury, Leicester, and Warrington in the 1891–93 epidemic, the correlation between vaccination and mildness of disease was + 0·7935, and in Glasgow as high as + 0·9123.

(2) The greater the amount of foveation, the greater is the diminution of the case mortality (see Table 184).

TABLE 184

	Admitted.	Died.	Percentage Mortality.
With 4 scars or more . . .	3,139	171	5·4
„ 3 „	1,902	171	9·0
„ 2 „	1,442	184	12·8
„ 1 scar	930	168	17·9

(3) The greater the area of cicatrix left by the vaccination, the greater is the diminution of the case mortality (see Table 185).

TABLE 185

Area of Scar.	Admitted.	Died.	Percentage Mortality.
Area of $\frac{1}{2}$ sq. in. or more . .	5,564	388	7·0
„ $> \frac{1}{3}$ sq. in. but $< \frac{1}{2}$ sq. in.	889	138	15·5
„ $< \frac{1}{3}$ sq. in.	933	158	16·9

According to Turner (1905–06), among cases with equal total areas of vaccination scarring there is no difference in the amount of protection afforded by different numbers of scars.

(4) In vaccinated persons the mortality from smallpox increases with the length of time elapsing since vaccination. Table 186 affords a comparison, in this respect, between vaccinated and unvaccinated persons. This table also shows a very different age-grouping of admissions in vaccinated and unvaccinated persons, strongly suggesting that vaccination affords protection against infection as well as against death.

TABLE 186

Years.	Vaccinated.			Unvaccinated and Doubtful.		
	Admitted.	Died.	Percentage C.M.	Admitted.	Died.	Percentage C.M.
–10 . .	143	2	1·4	1,441	459	31·9
11–20 . .	1,218	23	1·9	761	166	21·8
21–30 . .	2,675	144	5·3	374	129	34·5
31–40 . .	1,861	247	13·3	180	80	44·4
41–50 . .	893	174	18·2	102	57	55·9
51–60 . .	311	55	17·7	73	31	42·5

A striking piece of evidence in favour of the value of vaccination is afforded by the fact that of the 4,406 cases of smallpox in vaccinated persons in England and Wales during the period 1921–26 only 3 were under 12 years of age. This observation is difficult to explain except on the hypothesis that vaccination during infancy confers a large measure of protection against the disease lasting for some years. Vaccination performed immediately prior to exposure to smallpox (the incubation period of which is 12 to 14 days) affords almost absolute protection against the disease. Vaccination performed during the incubation period of smallpox either prevents the development of the disease, or predisposes to a modified form of the disease. Similarly, recent successful revaccination is said to afford an almost absolute protection against smallpox (Brownlee 1905–06).

In the United States there is a very interesting relationship between the vaccination laws in the different states and the incidence of smallpox (Table 187).

TABLE 187

RELATION OF SMALLPOX MORBIDITY TO VACCINATION LAWS IN THE UNITED STATES, 1919–28. (Woodward and Feemster 1933.)

Vaccination Laws.	No. of States.	Population.	No. of Cases.	Incidence per 100,000.
Compulsory vaccination	10	32,434,954	21,543	6·6
Local option	6	17,930,882	91,981	51·3
No vaccination laws	29	59,923,117	393,924	66·7
Compulsory vaccination prohibited .	4	4,002,888	46,110	115·2

The effect of vaccination appears to wear off with increasing age, as can be judged from Table 186; and it is therefore advisable, in countries where smallpox is rife, to perform revaccination every three years. In other countries vaccination

every five or ten years should suffice, provided revaccination is performed if the disease is introduced into the locality. Primary vaccination is, as a rule, best carried out during the first year of life. It should be deferred for at least 3 months after birth to enable the passive immunity received from the mother to wear off (Béclère 1936). (For general discussion of vaccination, see Greenwood 1930, 1935, Report 1931, Russell 1941, Robinson 1941.)

Complications of Vaccination.—Of the complications that may follow vaccination, suppuration is one of the commonest, and tetanus one of the most serious. Owing to the use of calf lymph, which cannot be satisfactorily sterilized, the introduction of micro-organisms into the wound is inevitable, but it is very difficult to say how often the calf lymph is responsible, and how often uncleanliness on the part of the operator, or during the after-treatment. Occasionally, as at Malmö, severe necrotic lesions may occur owing to the presence of pathogenic staphylococci in the lymph (Magnusson 1932, 1933). It may be noted that foot-and-mouth virus has been found occasionally in specimens of calf lymph, though there is no evidence that its presence had excited any unfavourable reaction in vaccinated children (see Gildemeister 1931, Gildemeister and Helm 1932*a*, *b*).

Tetanus is a very rare complication; thus in 1904–13 in the United States, 31 million lymph doses were inoculated into human beings, and only 41 cases of tetanus were observed. Infection in these cases may be carried by the lymph; but tetanus occurs only in those cases in which a protective shield or dressing strapped to the arm has been applied. Vaccination insertions treated openly have never been followed by tetanus (Armstrong 1927).

Generalized vaccinia is a serious complication, which is estimated to occur in 1 out of every 30,000 subjects vaccinated. Persons with chronic skin lesions are said to be specially susceptible. The disease varies from a mild rash with a few scattered vesicles and little constitutional disturbance to a severe variola-like disease with tracheitis, general glandular enlargement, widespread affection of the skin, and a rapid course to death. The case mortality is said to be 30–40 per cent. (see Ross 1941).

In recent years, a new complication has been observed, namely, *post-vaccinal encephalitis*. The first case studied in detail was observed by Turnbull in 1912; but it was not till the publication of Turnbull and McIntosh's report in 1926 that serious attention was drawn to the subject. Altogether in this country and abroad, some hundreds of cases have been observed, though the disease is now very much less frequent than formerly (Report 1936). Most of them have occurred after late primary vaccination. Vaccination during the 1st year of life rarely seems to be followed by encephalitis. Clinically, after an incubation period of 10 to 14 days, there is a rapid onset followed by an acute course, the symptoms being cerebral rather than spinal (Report 1928*a*). The morbidity is given by Ross (1941) as 1 in 100,000 cases, but it may be as high as 1 in 8,000 (see Fyfe and Fleming 1943); probably 1 in 50,000 is an average figure. The mortality, among 62 cases recorded by the Committee on Vaccination, was 58 per cent. Histologically, the lesions are widely distributed in the grey and white matter of the brain and cord, and are characterized particularly by extra-vascular parenchymatous infiltration with large endothelial and glial cells, and by perivascular areas of softening or demyelination (McIntosh 1928). The ætiology of these cases is as yet unknown. Greenfield (1928) suggests that, as the lesions are similar in many respects to those seen in encephalomyelitis following measles and in

disseminated sclerosis, the encephalitis is caused by some neurotropic virus which is capable of causing a latent infection, and may be stimulated into activity either by vaccination or by some exanthematous infection. This is the view favoured by the Committee on Vaccination (Report 1928*a*, 1930). McIntosh (1928), on the other hand, believes that post-vaccinal encephalitis is due to the vaccinia virus itself, because: (1) a similar encephalitis may occur in cases of smallpox; (2) the vaccinia virus has been recovered from the brain of cases of post-vaccinal encephalitis; (3) by the intracerebral injection of a neurotropic strain of the vaccinia virus, it is possible to produce in rabbits an encephalitis which he regards as essentially similar to that observed in human beings after vaccination. Each of these views has a substantial following (see Berger 1929, McIntosh 1930, McIntosh and Scarff 1930, Hurst and Fairbrother 1930, Clearkin 1930, Herzberg-Kremmer and Herzberg 1930, Eckstein 1931, Lucksch 1932, Armstrong 1932, Rivers *et al.* 1933, Ledingham 1934, Aldershoff 1935). (See also secondary encephalitis, p. 1921.)

The production of potent *antivaccinial sera* has now been shown to be possible (Ledingham *et al.* 1931, Fairbrother 1932), and there may be some call in the future for such sera, or for sera prepared against the variola virus, in the treatment of smallpox. Human convalescent blood or serum has been advocated by some workers, but this method of treatment is still in the experimental stage (see Yaoi 1935, Couzi and Kircher 1943). *Sulphonamides* are apparently without action on the virus itself, but they may be of value in the treatment of septic complications (Wilkinson 1942).

The Animal Poxes, including Avian Diphtheria or Roup

Pox occurs in a number of animals, such as sheep, goats, cows, horses, swine, fowls, and pigeons. The clinical manifestations vary; in its typical form it occurs as a vesico-pustular eruption on the skin, sometimes associated with very marked epithelial proliferation; on the other hand it may, as in horses, produce an eruption in the mouth (contagious pustular stomatitis), as well as on the skin; or, as in fowls, diphtheritic pseudo-membranes in the throat (avian diphtheria or roup). Histologically, inclusion bodies of the Guarnieri type are found in the lesions.

Whether the virus responsible for the animal poxes is identical with that responsible for vaccinia in man, or whether each virus is specific for its particular host is not yet clear, but evidence is rapidly accumulating to suggest that it is the vaccinia virus itself or some variety of it. Thus it is usually possible, by rabbit passage, to convert the mammalian pox viruses into a virus indistinguishable in its action on rabbits and human beings from the vaccinia virus (Toyoda 1924). Moreover animals inoculated with one variety of virus are more or less completely protected against another variety (see also Borrel 1903, Bosc 1903, de Jong 1917, Zwick 1924, von Wasielewski and Winkler 1925, Ledingham 1926).

Sheep-pox, known in France as *la clavelée*, is of some economic importance. It was studied by Borrel in 1903 who noted the occurrence of distinctive cellular bodies in the lesions, and showed that the causative agent was able to pass through coarse Chamberland filters. The virus has been cultured by Gins and Kunert (1937) on the chorio-allantoic membrane of the chick embryo.

The disease, which runs an acute febrile course, is characterized by the development of a vesicular eruption, later becoming pustular, on the more exposed parts

of the body. Infection can readily be transmitted to normal sheep by inoculation with the serous fluid contained in the loculated vesicles. The case mortality of the natural disease is very low. Post-mortem examination of fatal cases reveals the presence of generalized lesions. The lungs contain multiple, small, dense, spherical nodules, hyaline in appearance with a central opaque spot; they are commonest near the pleural surface. Similar, but less obvious, lesions are also seen in the liver and kidneys. Prophylactic inoculation with a sensitized vaccine is widely practised in France (Bridré and Boquet 1923, 1933). Inoculation with vaccinia virus is said to protect sheep against sheep-pox (Gins and Kunert 1937).

Rabbit-pox has recently been submitted to a careful experimental study by Pearce and her colleagues (Pearce *et al.* 1936*a*, *b*, Rosahn *et al.* 1936*a*, *b*, Hu *et al.* 1936). The virus is filtrable through Berkefeld V candles; it is antigenically related to the vaccinia virus, but not to Virus III (see p. 1909), or the virus of infectious myxoma (see p. 1978); and it is able to infect guinea-pigs, mice, calves, and probably rats.

Fowl-pox requires separate consideration. According to Doyle (1929) three clinical forms may be recognized: (1) wart-like nodules on the comb, wattles, and skin of the head; (2) adherent, yellow, cheesy membranes in the mouth; (3) a watery or muco-purulent discharge from the eyes and nose; this form is sometimes referred to as "roup." One, or any combination, of these forms, may be present in the same bird. These clinical forms have long been regarded as separate diseases, but the evidence now accumulating suggests that they are caused by one and the same filtrable virus, though the possibility that similar clinical conditions may be due to other agents cannot be excluded. Moreover, recent work suggests that the fowl-pox virus is related to the vaccinia virus. Thus Toyoda (1924) maintains that by passage through rabbits the fowl-pox virus can be converted into the vaccinia virus. Findlay (1928), however, has been unable to confirm this. The exact relationship between these two viruses must be left for the future to determine, but in any case the fowl-pox virus, both in its pathogenicity and in its serological reactions, seems to be less nearly related to the vaccinia virus than are the mammalian pox viruses. In the lesions of fowl-pox so-called Bollinger inclusion bodies, and Borrel elementary bodies, corresponding respectively to the Guarnieri corpuscles and the Paschen bodies in vaccinia, can be found (see Ludford and Findlay 1926).

Doyle and Minett (1927) found that pigeons were resistant to fowl-pox virus, but that fowls were susceptible to the virus of pigeon-pox. Moreover, they obtained evidence that pigeon-pox protected fowls against fowl-pox. Following on these observations, Doyle (1930) introduced the prophylactic vaccination of fowls with a vaccine consisting of dried pigeon-pox crusts suspended in glycerolated saline. This method is now being used on a wide scale with apparently very favourable results. The relationship of the two viruses is not very clear, but according to Doyle (1930) repeated passage of the pigeon-pox virus through fowls leads to an increase in its virulence for the latter; after 9 passages it may acquire all the characteristics of the typical fowl-pox virus.

Kikuth and Gollub (1932) have described a disease of canaries, which appears to be **canary-pox.** It has been studied by Burnet (1933) and Burnet and Lush (1936*a*), who find that the clinico-pathological picture differs from that of fowl-pox, but that the viruses of the two diseases are antigenically closely related.

One very interesting property of the pox viruses is their power to give rise in

certain hosts—sheep and fowls particularly—to tumours characterized by epitheliomatous proliferation, superficially resembling neoplasms. For a discussion on the relation of the animal pox viruses to vaccinia virus and to each other, the reader is referred to a review by Findlay (1936).

Contagious Pustular Dermatitis of Sheep

Contagious pustular dermatitis is a not infrequent malady amongst sheep in this country, and has been described by several workers on the Continent. (For refs. see Glover 1928.) It is probable that natural cases occur also amongst goats. The disease varies in severity: in the mild form there are pustular lesions on the lips and surrounding tissues; in the severe form lesions occur on the oral mucosa, the vulva, the cornea, and on various other parts of the body, particularly the regions of the coronet and the tail. Sheep of any age may be attacked, but the disease is commonest in weaned lambs up to 1 year old. Experimentally vesico-pustular lesions can be produced by scarification of the thighs of normal lambs, or by intradermal inoculation of the lip with a suspension of dried crusts from the natural lesions; the crusts generally prove active in a dilution of 1/50,000. All attempts to transmit the disease to laboratory animals have failed. Glover (1928) has brought evidence to suggest that the ætiological agent is a filtrable virus; he was able to show that Berkefeld V filtrates of scrapings of these fresh lesions, autolysed at 37° C. for 3 hours before filtration, or of the dried crusts submitted to tryptic digestion, were infective for normal lambs. The virus in the dried crusts resists desiccation for at least 4 months. Under natural conditions the disease appears to be highly infectious. Animals that have recovered from the disease possess a high degree of immunity, sufficient to protect them against re-infection for at least 8 months.

HERPES

Herpes simplex.—Herpes simplex or febrilis occurs in two forms. In the first form—often known as *herpes labialis* small vesicles, which are almost always bilateral, occur on the muco-cutaneous borders of the mouth or nostrils of patients suffering from a variety of diseases, of which pneumonia is the commonest. In the second form, the lesions occur in the mouth itself, constituting the disease referred to variously as *aphthous stomatitis*, ulcerative stomatitis, and acute infectious gingivo-stomatitis (see Black 1938). It affects mainly children and, like labial herpes, may recur from time to time. The observations of Dodd, Johnston and Buddingh (1938) showed that this second form, the ætiology of which had remained persistently obscure, was in all probability a primary herpetic infection. The further observations of Burnet and Lush (1939*b*) and of Scott and Steigman (1941) leave little doubt of the essential correctness of this conclusion. The disease should therefore be called *herpetic stomatitis*. Both forms of the disease tend to be recurrent.

Inoculation of the vesicular fluid on to the scarified cornea of a rabbit leads to the development of a severe keratitis, which can be propagated indefinitely by passage. If fluid is taken from an early keratitis in the rabbit, it is possible to produce with it specific lesions in a number of different tissues, such as the

skin, buccal mucosa, trachea, liver, adrenal, ovary, salivary glands, brain, and spinal cord (Goodpasture and Teague 1923–24*a*, Urbain and Schaefer 1927, Smith 1931*a*). Intracutaneous inoculation of human vesicular fluid produces in the rabbit an insignificant papular eruption, but once it has become adapted to the rabbit's tissues, the virus gives rise to a local herpetic lesion ; this method is of considerable value in the titration of infective material. Direct inoculation of human vesicular fluid on to the pad of the guinea-pig's foot is uniformly followed by the development of local vesicular lesions (Bedson 1934). A certain proportion of rabbits inoculated on the cornea develop, after a few days, a fatal encephalitis, characterized by widespread small-celled infiltrations, intense nerve-cell degeneration, and diffuse proliferative changes of some of the fixed elements of the tissue affected (da Fano 1923). Direct intracerebral or subdural inoculation of vesicular fluid into rabbits gives rise to a meningo-encephalitis which proves fatal in about 5 days, and which is transmissible in series to other rabbits by inoculation of the brain suspension.

Rabbit brain is highly infective, but its infectivity can be increased by homogenization —a process which probably acts by disintegration of the cells and liberation of the virus particles in a free form. Freshly homogenized virus may prove fatal on intracerebral inoculation into rabbits in 12–30 hours, and even one ten-millionth of a gram of infected brain tissue may kill the animals within 5 days (Buggs and Green 1936).

Experiments with monkeys have not given uniform results, but according to Teissier, Gastinel and Reilly (1925) cutaneous inoculation in *Callithrix* may result in the development of vesico-pustules, though often merely small papules appear. Corneal or intracerebral inoculations were without effect.

The virus can be passed from rabbits to mice by intracerebral inoculation with the brain of a rabbit suffering from herpes encephalitis, or with the ground-up nictitating membrane of a rabbit in which the corneal reaction is well established (Steigman and Scott 1942). Encephalitis develops in 3–10 days. Infection can be maintained indefinitely by passage through mice (Andervont 1929). With a neurotropic strain, cutaneous inoculation or intranasal infection of mice gives rise to encephalitis (Gildemeister and Ahlfeld 1936, Berry and Slavin 1943, Slavin and Berry 1943).

The herpes virus can be grown easily in tissue cultures, one of the most suitable media being rabbit testis (Parker and Nye 1925*b*, Gildemeister *et al.* 1929, Smith 1931*b*). It can also be adapted to grow on the chorio-allantoic membrane of the developing chick embryo. Since pocks are formed on the membrane, this method lends itself to the titration of virus and of neutralizing antiserum (Burnet 1936, Burnet and Lush 1939*a*, Shaffer and Enders 1939). In the vesicular fluid the virus is present in the form of elementary bodies, which can be stained by suitable means (Taniguchi *et al.* 1934). Examination of the corneal lesions in rabbits during the first 24 hours reveals the presence of large numbers of acidophilic intranuclear inclusion bodies—referred to as " α-bodies " by Lipschütz (1921) (Fig. 301). The diameter of the virus particles themselves is about 125 mμ. Though the virus may be demonstrated in the blood after intratesticular, and occasionally after intracutaneous, inoculation of rabbits (Gildemeister and Heuer 1929), the work of Goodpasture and Teague (1923–24*b*) seems to show that it may enter the peripheral nerves—sensory, motor, or sympathetic—from a local lesion and reach the central nervous system, where it is liable to give rise to a myelitis or encephalitis (see also Burnet and Lush 1939*c*, Berry and Slavin 1943).

Rabbits that have passed through an attack of experimental keratitis possess a considerable degree of immunity to subsequent corneal, or even intracerebral, inoculation (Levaditi and Nicolau 1922). This immunity appears to depend largely on the development of humoral antibodies (Bedson and Crawford 1927, Schultz and Hoyt 1928). Bedson and Bland (1929) were able to obtain specific complement fixation with the herpes virus in the presence of serum from the hyper-immunized guinea-pig. Antibodies may also be demonstrated in a considerable proportion of children and adults, either by a specific neutralization test (Zinsser and Tang 1929, Andrewes and Carmichael 1930), or by complement fixation (Brain 1932). Burnet and Lush (1939*b*) and Burnet and Williams (1939), working in Melbourne, found neutralizing antibodies present in over 90 per cent. of children and adults of the hospital classes, but in only about 50 per cent. of members of the higher social classes. This they explain by the greater frequency of primary herpetic stomatitis in early life among children of the lower classes. Since recurrent herpes affects persons whose blood serum contains neutralizing antibodies, it is assumed that the virus persists in the body tissues—probably in the mouth (Scott and Steigmann 1941)—and that immunity is to a considerable extent non-specific. The failure of the antibody to afford protection is curious. Herpes febrilis constitutes, in fact, one of the most striking exceptions to the general rule that filtrable virus infections usually leave behind them a solid and lasting immunity. A skin reaction that is said to be positive in persons subject to recurrent attacks of herpes has been described by Nagler (1944).

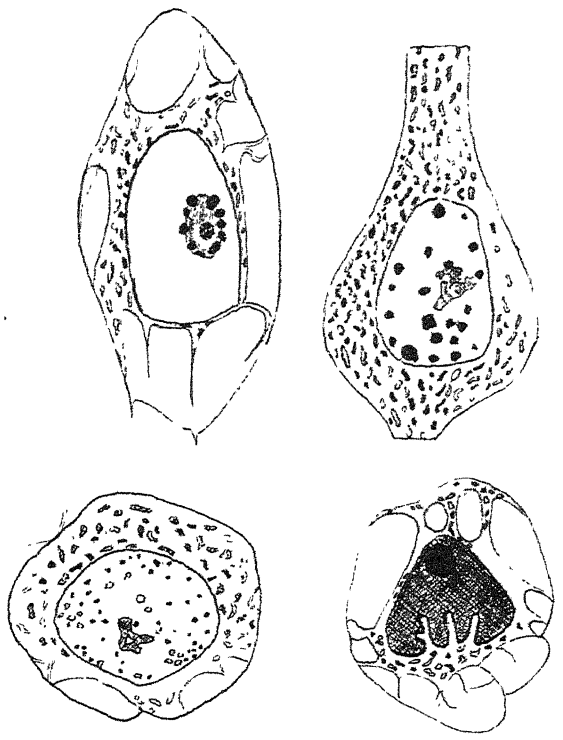


FIG. 301.
Intranuclear inclusion bodies in nerve cells of rabbits experimentally infected with herpes febrilis.
(After Cowdry and Nicholson 1924.)

Keratitis herpetica of human beings was shown by Grüter in 1912–14 to be transmissible to rabbits by inoculation of the scarified cornea. This observation was confirmed by Luger and Lauda (1921), who found, histologically, alterations in cellular structure similar to those in herpes febrilis. These consisted of giant-cell formation, the characteristic ballooning degeneration of Unna, and bodies which they considered to be due to a specific degenerative reaction of the nucleus, but which Lipschütz regards as inclusion bodies.

Herpes genitalis has likewise been shown to be transmissible to rabbits, but the keratitis set up by the genitalis virus is much less severe than that set up by the febrilis virus. Similarly, on subdural inoculation the genitalis virus is less virulent; by this route the febrilis virus uniformly produces encephalitis in rabbits, fatal in 4 to 6 days; the genitalis virus does not uniformly produce encephalitis, and, when it does, the rabbits do not die for 5 to 17 days (Blanc and Caminopetros 1924). Lipschütz (1921) succeeded in transferring genital herpes to human beings. Both in the human lesions and in the rabbit's cornea, he found nuclear inclusion bodies of the so-called β-type present in large numbers; they are best looked for in the rabbit's cornea on the 3rd day after inoculation. He was unable to produce immunity to the genitalis virus by inoculation of the rabbit's cornea with the febrilis virus. He therefore regards the two viruses as distinct.

Herpes zoster.

Herpes zoster differs clinically from herpes febrilis in forming large vesicles or bullæ, in occurring on the trunk, in being almost always unilateral, in being accompanied by sharp neuralgic pains, in being distributed along the course of sensory nerves, and in being non-recurrent. Experiments made to ascertain whether zoster is transmissible to animals have given most conflicting results.

Lipschütz (1921) claimed to have produced keratitis in rabbits with material from 3 out of 7 cases of zoster. Both in human lesions and in the rabbit's cornea, he found acidophilic nuclear inclusion bodies, which he calls "zoster bodies." In the rabbit's cornea these bodies were present in greatest numbers on the 4th day after inoculation. The keratitis, when it did develop, was mild, and consisted of small round vesicles, which did not appear till the 4th day, and which after a few days tended to regress. Kundratitz (1924–25) claimed to have transmitted zoster to infants; in contradistinction to herpes febrilis, in which the incubation period of the experimental human disease is only 24 to 48 hours, the incubation period in zoster was 12 to 15 days. Teague and Goodpasture (1923–24) obtained some evidence that the zoster virus differed from that of herpes febrilis mainly in its degree of virulence, but their experiments were not very satisfactory.

Cole and Kuttner (1925) failed to confirm the work of either Lipschütz, or Teague and Goodpasture. Using material from 9 cases of zoster, they performed corneal and intracerebral inoculations on rabbits; they inoculated *rhesus* monkeys by the cerebral, cutaneous, neural, and corneal routes, and vervet monkeys by the testicular route; and they rubbed the material on to the scarified tar-painted skin of rabbits and guinea-pigs —all with negative results. Since herpes febrilis can be easily transmitted to rabbits, while zoster either cannot be transmitted at all or only irregularly, they conclude that the two diseases are ætiologically distinct. Seidenberg (1931) was likewise unable to produce keratitis or orchitis in the rabbit with vesicular fluid from zoster patients; and the cornea of rabbits inoculated with zoster virus was found to be still susceptible to the virus of herpes febrilis.

More recently Goodpasture and Anderson (1944) have succeeded in growing the zoster virus on pieces of human skin grafted on to the chorio-allantois of the developing chick embryo. Microscopic lesions becoming pustular were produced on the skin under these conditions, though not true vesicles; and intranuclear acidophilic inclusion bodies were demonstrable in the affected epithelial cells. The chicken-pox virus, it may be noted, could not be cultured in this way.

Elementary bodies may be demonstrated by suitable staining methods in zoster vesicles, particularly during the first 48 hours (Amies 1934, Taniguchi *et al.* 1934). It seems probable that they constitute the specific virus of the disease.

Summarizing, it would appear that the viruses of herpes febrilis, genitalis, and zoster all differ from each other; the febrilis virus is the most virulent for the rabbit and the zoster virus the least. Whether they are to be regarded as distinct species of virus, or as varieties of the same species, it is at present impossible to say.

For the relation of herpes zoster to varicella see below.

VARICELLA

Chicken-pox appears to be due to a virus, which can be demonstrated by suitable staining methods in the vesicular fluid (Aragão 1911, Paschen 1919, Taniguchi *et al.* 1932*b*). The virus particles, or elementary bodies, are of coccoid form, and are arranged singly, in pairs, or in short chains. The virus is of very low infectivity for animals. The disease is characterized histologically by the presence of intranuclear acidophilic inclusion bodies in the epithelial cells (Tyzzer 1906), indistinguishable from those described by Lipschütz (1921) in zoster. Rivers and Tillett (1923, 1924*a*, *b*) and Taniguchi and his colleagues (1932*b*) reported transmission of the virus to the rabbit's testicle, but subsequent observations leave little doubt that they were working, not with varicella virus, but with the so-called Virus III (see p. 1909). On the other hand, the presence of acidophilic intranuclear inclusion bodies in the testicles of vervet monkeys inoculated with material from human chicken-pox lesions were probably the result of infection with the true varicella virus (Rivers 1926, 1927).

Amies (1933) has described a specific agglutination reaction, using as antigen a 0·25 per cent. formolized suspension of elementary bodies prepared by the differential centrifugation of human vesicular fluid. Of 55 sera taken from patients 2–12 days after the onset of the attack, 35 contained agglutinins in a titre of 1/4–1/256, while 20 gave negative results. Antibodies appear about 5 days after the onset, and persist for some considerable time. Convalescent serum has apparently no prophylactic value (Lewis *et al.* 1937).

Relation between Herpes zoster and Varicella.

A large body of clinical evidence has been produced suggesting that zoster and varicella are closely allied diseases. The experimental evidence in favour of this conception has been summarized by Lipschütz and Kundratitz (1925) as follows: (1) In infants inoculated with zoster material, apart from the local vesicles that develop, a general varicella-like eruption may occur; (2) children brought into contact with infants inoculated with zoster may develop chicken-pox; (3) inoculation experiments with zoster, made on children who have recovered from varicella, prove negative; (4) inoculation experiments with varicella, in children who have recovered from natural or experimental zoster, prove negative; (5) the serum from patients convalescing from zoster has a protective action against varicella; (6) both varicella and experimental zoster have the same incubation period; (7) similar cytological appearances are found in both diseases.

On the other hand, there are a number of differences (Barrett 1938). For example, zoster is not contagious: chicken-pox is highly contagious. A leucopenia with a relative mononucleosis is the rule in chicken-pox, but not in zoster. Chicken-pox affords almost complete immunity against chicken-pox, but not against zoster. Zoster affords almost complete immunity against zoster, but not against chicken-pox. Chicken-pox is much commoner in zoster contacts than zoster is in chicken-pox contacts.

The close relationship of the two diseases to each other is supported by the observations of Netter and Urbain (1924, 1926, 1931), who found apparently identical antibodies, demonstrable by the complement-fixation test, in the serum of patients with zoster and varicella. Brain (1933), moreover, obtained equally good fixation with zoster and with varicella vesicular fluids. It has further been shown that serum from convalescent zoster patients often agglutinates a suspension of varicella elementary bodies, though to a rather lower titre than a suspension of zoster elementary bodies. On the other hand, Hasskó, Vámos and Thoroczkay (1938) never obtained complete cross-fixation of complement between varicella and zoster sera; and in a high proportion of sera no cross-fixation occurred at all. As already noted, Goodpasture and Anderson (1944) were able to cultivate the zoster virus on pieces of human skin that had been grafted on to the chorio-allantois of the developing chick embryo, but failed to grow the chicken-pox virus under these conditions. The evidence, on the whole, suggests that the two diseases are caused by viruses that are closely related to each other, but are probably not identical.

Epidemic Keratoconjunctivitis

The inclusion of epidemic keratoconjunctivitis among the filtrable virus group must, for the present, be tentative. According to Smitmans (1940), this disease was recognized in Vienna as long ago as 1889. It has been known in India for several years, but it assumed special prominence during the second world war, when it was reported from Germany (Smitmans 1940) and from the United States (Sanders 1942, Rieke 1942). It was very common in shipbuilding yards. It is characterized by redness, swelling and sometimes vesiculation of the conjunctiva. There is a relative absence of conjunctival discharge; what there is, is sero-mucoid, seldom purulent. The disease is mainly unilateral, but the second eye often becomes affected. In about half the cases inflammation extends to the cornea, but corneal scars are said to be left in only 1 to 10 per cent. of the patients. Nasal catarrh is sometimes present at the onset, headache and malaise are common systemic manifestations, and enlargement of the homolateral pre-auricular gland is almost invariable. The disease lasts, as a rule, about 2 to 4 weeks, and is irresponsive to treatment. No pathogenic bacteria have been isolated, but the observations of Sanders (1942), Sanders and Alexander (1943), and Sanders, Gulliver, Forchheimer and Alexander (1943) suggest that the disease is of virus origin. These workers record the isolation of a virus from two patients. At first it could be maintained only in tissue culture, but later it was adapted to the mouse brain. Intracerebral inoculation of mice gave rise in 2 to 7 days to a disease characterized by lethargy, tonic and clonic convulsions, sometimes spastic paralysis, and death within 24 hours of the onset of symptoms. The virus passed through collodion membranes of 75 but not of 50 mμ, suggesting that it was probably about 25 mμ in diameter. It was neutralized by serum from convalescent patients. Inoculated into the eye of a human volunteer, the mouse virus produced a mild but characteristic attack of keratoconjunctivitis, followed by the appearance of neutralizing antibodies in the patient's serum. The disease does not appear to be highly contagious from patient to patient, but it is readily transferred by indirect means. Rigid precautions are necessary to prevent infection from one patient to another by instruments and hands in the surgery. Patients should be isolated, and contacts kept under observation. Intravenous inoculation of human convalescent serum is said to be of benefit (Braley and Sanders 1943).

FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease is a highly infectious disease of cloven-footed animals. Cattle, pigs, sheep, and goats are chiefly affected; reindeer and camels are susceptible. Occasionally human beings contract the disease (see Flaum 1939). Epidemics occur from time to time in most countries, the disease remaining endemic in the intervening periods. In this country the disease is not strictly endemic, but outbreaks occur from time to time, occasionally reaching epidemic proportions. In the year 1923, for example, 1854 separate outbreaks were reported, necessitating the slaughter of 125,098 animals.

The disease is usually mild, causing a mortality in cattle of not more than 2–3 per cent., and a slightly higher mortality in sheep and pigs; on the Continent, however, a malignant form sometimes occurs, causing in young animals a mortality of 50–70 per cent. The importance of the disease lies not so much in the deaths to which it gives rise as in the debilitating effect it has on the general health of the animals, leading to a fall in milk and meat production. The incubation period is 2 to 7 days. Infection usually occurs from animal to animal, the infective material coming into contact with the mucosa of the mouth, nose or conjunctiva, or with an abraded skin surface. Cases of direct infection are easy to understand; the real problem lies in explaining the origin of foot-and-mouth disease in animals that have not, so far as can be ascertained, been in contact with infected animals. Numerous explanations have been offered of these cryptogenic cases. Thus it is supposed by some that infection is carried by contaminated hay, straw, milk, drinking water, clothes, and other objects. In support of this, it has been found that the virus may survive on chopped hay at room temperature for at least 15 weeks, and on bran for at least 20 weeks (Report 1927). Moreover, there is evidence that the virus may resist putrefaction for long periods (Report 1928*b*). In this way it is possible to understand how infection may lie dormant for months in dried discharges or in carcasses of infected animals. That the virus may not only remain alive, but may keep its virulence under these conditions, was shown by the development of foot-and-mouth disease in a calf fed with hay that had been contaminated 1 month previously with infected saliva, and in pigs fed on crushed bones from the frozen carcasses of infected animals that had been killed several weeks previously (Report 1927).

Another explanation is that the virus is carried by human and animal agencies. Thus it has been suggested: (1) That birds, particularly starlings, may carry infectious material on their feet; for this there is no direct evidence (see Bullough 1942). (2) That wild rodents may carry the disease and transmit it to cattle, sheep, and pigs coming into contact with them (see Beattie *et al.* 1928). Many of these rodents are susceptible to experimental infection, but practically all experiments designed to test the possibility of natural spread amongst them have given negative results. The virus has, however, been demonstrated in hedgehogs on an infected farm. Experimentally, it has been found that healthy hedgehogs placed in contact with affected cattle may themselves become infected, and that a healthy cow placed in a stall with infected hedgehogs may develop typical foot-and-mouth disease (Report 1937). (3) That the virus may gain access to, and persist on, the nasopharyngeal mucosa of human beings, who can then act as healthy carriers of the disease; for this there is a certain amount of

epidemiological, but no direct experimental evidence (Kling and Höjer 1926). (4) That infected cattle may remain as chronic carriers; it has been found that infective material may persist on the hoofs for some time after recovery from the disease, but this appears to be an exceptional occurrence (Gins and Krause 1923). Animals that have recovered from the disease may excrete the virus in the urine, but how often urinary carriers occur is still in doubt (Waldmann *et al.* 1931). The experimental evidence so far collected does not favour the view that chronic carriers are common. On the other hand, there is a considerable amount of epidemiological evidence to suggest that infection is introduced into a herd by means of apparently healthy animals coming from another herd. Moreover, if the virus could persist on the healthy mucosa of animals, or remain latent in their tissues, it would not be difficult to understand how outbreaks of the disease might occur in herds that had had no demonstrable contact with the disease. At the present time it is impossible to come to any definite conclusion on the most common method of indirect infection; but the Foot-and-mouth Disease Research Committee (Report 1928*b*) favour the view that the persistence of the virus in dried discharges on fodder, hair, and other materials, and in carcasses, is of greater epidemiological significance than its carriage in the body of recovered animals.

Experimental Reproduction of the Disease.—The disease can be experimentally reproduced in cattle, sheep, and pigs by rubbing infective material from the foot-and-mouth lesions into the scarified mucosa of the lip or mouth. Intramuscular inoculation requires a larger dose, and the effect is less regular.

In *cattle* inoculated intramuscularly, the temperature rises in 1 to 3 days to about 104° F., and the characteristic lesions appear in 1 to 5 days. Of 71 cattle that reacted to inoculation, foot and mouth lesions occurred together in 63; in the remaining 8, mouth lesions were absent (Report 1927). Great differences in the severity of the disease occur as the result of inoculating different strains of virus. Sheep inoculated intramuscularly generally show a rise of temperature to 104° or 105° F. in 18 to 48 hours. Foot lesions are constant, appearing in 1 to 3 days or so, depending on the virulence of the strain. Mouth lesions are uncommon. Pigs inoculated intramuscularly show a rise of temperature in 20 to 48 hours; foot lesions constantly develop, generally in 1 to 3 days; mouth lesions are not quite so constant, and do not appear till after 3 days.

Waldmann and Pape (1920) showed that the disease could be reproduced experimentally in *guinea-pigs* by cutaneous or subcutaneous inoculation into the balls of the fore and hind feet, or into the hairless side of the metatarsus. Not all strains, however, seem to be capable of infecting these animals.

After intracutaneous inoculation of the foot pad, there is an incubation period lasting from 12 hours to 4 days. The first signs of reaction are a rise of body temperature, and redness and swelling of the foot, followed by the development of small vesicles radiating from the line of inoculation. The vesicles coalesce to form a large tense blister over the entire sole, containing as much as 0·1 ml. of semi-transparent fluid, which is rich in virus. General symptoms of illness occur, and secondary vesicles develop on the uninoculated feet, and on the tongue, lips and gums. About 2 days after their appearance, the vesicles become greyish-white and flattened, and then gradually dry up; the whitish colour of the skin may remain for some time. The vesicles usually heal completely in 10 to 14 days. Though the animals are often ill, refuse food, and lose weight, only about 5 per cent. of them die. The remainder recover, and are henceforth highly resistant to fresh inoculation. The disease can be reproduced by intramuscular, intraperitoneal, and intravenous inoculation. After intravenous injection, vesicles develop on the legs in 3 to 4 days; mouth lesions are not usually observed (Waldmann and Pape 1920).

Maitland (1928) has brought evidence to suggest that mechanical pressure is to some extent responsible for the localization of the lesions on the pads of the feet after intramuscular injection of the virus. She found that if one foot was adequately protected from pressure by being wrapped in cotton-wool, no lesions developed on this foot after intramuscular injection, though typical vesicles appeared on the unprotected feet. Moreover, by exposing the feet to unusual pressure, as by keeping the animals in cages with a coarse wire-mesh floor, she was able to induce the formation of vesicles on the toes, and even on the dorsal parts of the feet—situations in which vesicles do not usually occur. The disease can readily be passed from animal to animal by inoculation with infective material. Complement-fixing bodies may be found in the blood serum of convalescent guinea-pigs (see Krag and Schmidt 1937).

Rabbits, rats and mice can all be infected, but the experimental disease is less severe and less typical than in guinea-pigs; dogs and cats sometimes prove susceptible; ducks can be infected by inoculation of the virus into the thickened pads of skin under the toes (Report 1937). The disease practically never spreads from experimentally inoculated to normal rodents. Even highly susceptible cattle placed in contact with infected guinea-pigs rarely contract the disease.

Properties of the Virus.—The virus is present in great concentration in the vesicle fluid of experimentally infected guinea-pigs; even when diluted 1/1,000,000 times it is generally infective for normal guinea-pigs. In the blood, during the febrile stage of the illness, the concentration is less, but even this is active in a 1/1,000 dilution (Report 1927). In cattle during the febrile stage of the disease, the saliva, urine, milk and vesicular fluid are infective; but in 3 to 6 days after the appearance of the lesions they lose their infectivity completely. For diagnostic purposes, it is therefore important to use fresh vesicular material, not more than 2 days old (Olitsky *et al.* 1928). The rapid disappearance of the virus from the local lesion and blood of infected animals is one of the most remarkable features of foot-and-mouth disease; within the space of 3 or 4 days as a rule the virus, from being present in large quantities, is reduced to such an extent that it is no longer demonstrable by experimental methods.

Loeffler and Frosch (1898) in 1897 were the first to show that the foot-and-mouth virus was filtrable. When present in diluted vesicle fluid, it will pass readily through Berkefeld, Chamberland and Seitz filters. The size of the virus is still in process of determination. Using the gradocol membrane technique, Galloway and Elford (1933) found the average particle diameter to be 8–12 $m\mu$. Centrifugation experiments, however, placed it at 20–25 $m\mu$ (Elford and Galloway 1937, Schlesinger and Galloway 1937). Barnard (1937), studying photomicrographs taken by ultra-violet light, found the diameter to be even larger—about 40 $m\mu$. The organisms tended to be rod-shaped, and the optical system used may have been incapable of resolving the smaller diameters completely; the breadth of the virus may therefore have been rather less than the figure quoted. Further work must be awaited before the discrepancy between these three estimates can be explained; but it may be noted that the gradocol membrane technique will necessarily afford a measurement of the smallest diameter of the virus—just as with spirochætes—and that the measurement afforded by centrifugation is determined by the density value, which itself is to some extent a matter of assumption. The suggestion that the A, B, and C varieties are not identical in size (see Modrow 1929) finds no support in the observations of Galloway and Elford (1937). The virus was successfully cultivated by Maitland and Maitland (1931) in tissue cultures containing guinea-pig embryo tissue and clotted guinea-pig plasma. Seventeen

successive subcultures were observed, and a titre as high as 1/100,000 was obtained (see also Hecke 1930, Striegler 1933, Köbe and Fertig 1936.

The virus is very resistant to certain methods of disinfection, though easily destroyed by others. Thus when vesicle fluid from the guinea-pig is dried rapidly on a glass slide at 37° C., it is often inactivated immediately; but if dried slowly at room temperature, and kept over H_2SO_4, it survives for 3 to 6 months. Filtered vesicle fluid is destroyed by moist heat at 55° C. in 15 to 40 minutes. The virus when present in dilute vesicle fluid is readily destroyed by high dilutions of chlorine, iodine, potassium permanganate, caustic soda, and sulphuric acid, but in epithelial scrapings it is much more resistant. Its apparent resistance to chemical disinfectants in the presence of organic matter is probably due to the protective effect exerted by the protein coagula formed in the substrate (Olitsky *et al.* 1928). If coagulation is prevented, or if disinfectants are used that do not coagulate protein, the virus is found to be very susceptible. In buffer solutions the virus shows two zones of stability—an optimal zone at pH 6·5–10·0, and a less favourable zone at pH 2·5–3·5 (Pyl and Klenk 1933, Galloway and Elford 1935). At a suitable pH it may survive in the ice-chest for a year.

Plurality of Various Types.—Immunological experiments have shown that there are at least three different types of foot-and-mouth virus. Vallée and Carré (1922) met with two types, Virus O from the Department of the Oise, and Virus A from Germany (*origine allemande*). Both viruses were equally pathogenic for cattle, pigs, and sheep, and gave rise to the same clinical symptoms. Animals that had recovered from infection with one strain proved immune to further infection with the same strain, but were readily infected by the other strain. Waldmann and Trautwein (1926), in an examination of 30 strains of different origin in Germany, encountered three types; 4 strains belonged to Type A, 4 to Type B, and 5 to Type C; the remaining 17 strains were variants of the main types. Strains of different types were found in the same area. Oxen and guinea-pigs were infected experimentally with the three different types in succession. According to the Foot-and-mouth Disease Research Committee (Report 1928*b*), Vallée's Type A is the same as Waldmann's Type B, and Vallée's Type O the same as Waldmann's Type A; Waldmann's Type C is a new type. The three types are said to be distinguishable by complement fixation (Ciuca 1929, Helm 1933).

Further observations, particularly by Manninger and von László (1930), Trautwein and Reppin (1932) and Daubney (1934), have cast considerable doubt on the fixity of these three types. Variant and intermediate types have been observed, and one intermediate strain was found to pass into an O type as the result of guinea-pig passage and into an A type as the result of storage in the cold. Daubney regards the bovine virus as a labile organism subject to spontaneous phase variations. This interpretation is borne out by observations in Great Britain (Report 1937). The epidemiological importance of multiple types is obvious, since it renders possible the re-infection of animals that have already passed through one attack of the disease.

Immunity and Prophylactic Treatment.

An animal that has passed through an attack of the disease acquires a varying degree of immunity to the specific type of infecting virus. In cattle, subsequent resistance to infection appears to be very strong, persisting for some years; but pigs develop a much lower degree of immunity and can often be re-infected within a year (Report 1937). The serum of immune animals contains antibodies capable of neutralizing the virus *in vitro*. Prophylactically, immunity may be produced

by the injection of animals with a weakly virulent virus, given either alone or in combination with immune serum. The practice of using living virus is open to several objections.

Vallée, Carré and Rinjard (1926) succeeded in rendering cattle immune by the use of a formolized vaccine, prepared by exposing the virus to a concentration of 0·5 per cent. formol for 4 to 7 days at room temperature. Heifers inoculated subcutaneously with 10 ml. of Virus A and 10 ml. of Virus O vaccine were protected against subsequent infection with either strain, though one animal proved susceptible to Virus O. Immunity can likewise be produced in guinea-pigs by use of a formolized vaccine, but the immunity is only partial, and wears off in about 2 months; moreover, antibodies are not demonstrable in the blood serum. Guinea-pigs may, however, be rendered highly resistant by the intramuscular injection of living virus, after preliminary immunization with formolized virus; and in animals so treated antibodies may readily be demonstrated in the blood (Report 1927, 1928*b*). It remains true, however, that no satisfactory vaccine for the active immunization of cattle has yet been devised (Waldmann and Reppin 1935). Formolized vaccines have not had much success in the field (Waldmann and Reppin 1935); but more recently Waldmann (1938) has described a promising vaccine in which the virus is adsorbed on to aluminium hydroxide and treated with 0·05 per cent. formol for 2 days at 25° C. Results of large field trials are still awaited.

Methods for dealing with an outbreak of foot-and-mouth disease vary in different countries. In Great Britain, the United States, and Scandinavia a rigorous policy of stamping out the disease by slaughter and disinfection is practised. In Germany the system known as Ring-Impfung is officially employed. This consists in isolating the affected farms, and giving all contact animals combined active and passive immunization, using for this purpose living virus and polyvalent hyperimmune serum. The result is a mild attack of the disease, which leaves behind it a more or less lasting immunity. At the same time animals on surrounding farms are given an injection of serum, the effect of which is to afford them some degree of passive immunity for about 10 days. The serum may, however, mask a mild attack, so that the infection of some animals passes unrecognized and may form a starting point for further spread. The whole area is isolated for 2–3 weeks. The merits of the two policies have often been discussed. There is no doubt that the slaughter method has proved, on the whole, satisfactory in countries where it has been thoroughly carried out. It is difficult to judge the value of the isolation and immunization policy without knowing how often and how long animals may remain carriers of the virus after an attack of the disease. Immune polyvalent serum made by hyperimmunization of cattle against the three main types of virus may be used prophylactically or therapeutically. Its value, however, is limited. It may protect cattle and pigs exposed for only 3 hours or so to contact infection, but not those exposed for 24 hours (Report 1937). In guinea-pigs the serum, though usually unable to protect completely against the disease, may prevent generalization of the infection.

Vesicular Stomatitis of Horses

This disease is similar in many respects to foot-and-mouth disease. It has been observed chiefly in South Africa and the United States, and is characterized by the development of vesicles on the oral mucosa, particularly on that of the tongue. Though the disease is primarily one of equines, cattle may become infected naturally; the lesions in cattle resemble those of foot-and-mouth disease, but are less extensive, and do not usually affect the feet. Intramuscular injection of cattle with the

vesicular stomatitis virus does not produce lesions, whereas injection with the foot-and-mouth virus does ; in horses, on the other hand, inoculation of the scarified tongue with the stomatitis virus produces lesions, whereas the foot-and-mouth virus does not. Pigs do not contract vesicular stomatitis naturally, but can be infected experimentally. The guinea-pig is susceptible to experimental inoculation with the stomatitis virus. According to Olitsky, Traum and Schoening (1928) there is no cross-immunity between foot-and-mouth disease and vesicular stomatitis in cattle. The causative virus differs from that of foot-and-mouth disease in being larger (70–100 mμ by ultrafiltration, 60 mμ by centrifugation, 80–100 mμ by ultra-violet photography), in being spherical instead of rod-shaped, in multiplying readily on the chorio-allantoic membrane of the developing chick embryo, and in having only one zone of pH stability, limited on the acid side by pH 4·5 (Galloway and Elford 1933, 1935, 1937, Elford and Galloway 1937, Burnet and Galloway 1937, Barnard 1937). There are at least two antigenic types.

Vesicular Exanthema of Swine

A disease bearing a close resemblance to foot-and-mouth disease was recognized in California about 1932, and was called vesicular exanthema of swine by Traum (1936). Outbreaks occurred irregularly, but on a generally increasing scale, till in 1939–40 the number of infected premises was 123 and the number of animals on these premises was over 220,000 (White 1940). It is an acute febrile disease, characterized by vesicles on the snout, lips, oral mucosa, tongue, and feet. The vesicles are 2–3 mm. and upwards in diameter. There is often considerable systemic disturbance, the temperature reaching 108° F. The animals suffer from varying degrees of lameness. Growth is seriously interrupted. The case mortality is low except in young sucking pigs, which may die either as the result of cessation of lactation in the nursing sow or of mechanical obstruction to respiration by the vesicles. Animals that recover develop a temporary immunity, which passes off in a few months' time. The disease spreads readily by contact. The incubation period is about 48 hours. Experimental inoculation is successful by the intradermal or intramuscular route. Clinically, the disease cannot be distinguished with certainty from foot-and-mouth disease, and reliance in the first place has to rest on animal inoculation. As will be seen from Table 188 the virus of vesicular exanthema of swine differs from that of foot-and-mouth disease in being pathogenic to horses, but not to cattle or guinea-pigs.

TABLE 188

Differential Susceptibility of Animals to Inoculation (Traum 1936)

Virus.	Swine.	Cattle.	Guinea-pigs.	Horses.
Foot-and-mouth . . .	+	+	+	−
Vesicular stomatitis . .	+	+	+	+
Vesicular exanthema . .	+	−	−	±

+ = susceptibility. ± = susceptibility less and irregular.

The susceptibility of the horse to vesicular exanthema is less than that of cattle, and may perhaps vary with different strains of virus. The British workers (Report 1937) failed in their efforts to infect horses with a strain of Californian origin.

The virus is antigenically different from any of the known types of foot-and-mouth virus, and there appears to be no cross-immunization between the two viruses. In control of the disease reliance is placed on rigid quarantine measures.

Virus III Infection of Rabbits

Virus III was the name given by Rivers and Tillett (1923, 1924*a*, *b*) to a filtrable virus encountered in rabbits during experimental work on chicken-pox. Injected intratesticularly into rabbits it produces a high fever and characteristic lesions in the cornea, testicles and skin ; within the epithelial and endothelial cells of these lesions acidophilic nuclear inclusion bodies are found, similar to those seen in herpes and varicella. Injected intracerebrally it sometimes gives rise to a meningo-encephalitis, not unlike that of herpetic encephalitis (Rivers and Stewart 1928). Frozen and desiccated, and kept in sealed tubes on ice, it retains its virulence indefinitely ; even after 15 months under these conditions it was still active. Its activity is also maintained for at least 6 weeks if infected testicular suspensions are mixed with equal amounts of glycerol, and stored on ice. It is destroyed by heat at 55° C. in 10 minutes. Rabbits injected with the virus develop an immunity to subsequent inoculation, which lasts for 6 months or more. The serum of immune rabbits contains antibodies capable of neutralizing the virus either *in vitro*, or locally in the rabbit's skin when the serum and virus are inoculated together. Attempts to confer passive immunity on rabbits, by intravenous injection of immune serum 24 hours previous to inoculation of the virus, proved negative. Rivers and Tillett found that about 15 per cent. of their young stock rabbits were refractory to infection with Virus III, indicating that they had suffered spontaneously from the disease. The same virus was met with in rabbits by Miller, Andrewes and Swift (1924). According to Andrewes (1929), it can be grown in tissue cultures prepared from adult rabbit testis. Typical intranuclear inclusion bodies are formed in these cultures. The symptoms of natural Virus III infection in rabbits are unknown. The virus is brought to light only by serial intratesticular passage, preferably at 4-day intervals. It may sometimes be present in rabbit tumours (Andrewes 1940), being carried as a so-called " passenger virus."

INFECTIOUS ECTROMELIA

This is a highly infective disease of mice, which was first described by Marchal (1930) at Hampstead. It occurs in an acute form characterized by the presence of visceral lesions, in a chronic form characterized by necrosis of the feet, and in a latent symptomless form. In the chronic form one of the feet becomes swollen and œdematous, and serous fluid escapes from the surface, forming minute scabs. The disease may retrogress, in which case the foot becomes gangrenous and sloughs off leaving healthy tissue behind ; or infection may spread to another foot, the tail, or the mouth, and lead to the death of the animal. In the acute form the animals die without any external lesions. Post mortem the liver may be dirty grey in colour, or it may be red and mottled due to the presence of small white focal areas of necrosis. The spleen is slightly enlarged, and may show large white areas, especially in the more chronic cases. There is often an excess of pleural fluid. It must be emphasized, however, that mice may die of ectromelia without showing any definite macroscopic lesions. Histologically, the chief characteristic is necrosis of the mesoblastic tissues, and the presence in the epithelial cells of the skin and

intestine of large acidophilic cytoplasmic inclusion bodies (see Figs. 234 and 235, p. 958). The disease can be readily transmitted to normal mice by contact, and by inoculation with infective material. Rats are susceptible, but develop only an "inapparent" infection (Burnet and Lush 1936*b*).

The causative agent is a virus, 100–150 mμ in diameter, which can be demonstrated microscopically in the liver and cutaneous tissues (Barnard and Elford 1931). It is widely distributed throughout the body. It is destroyed by exposure to 55° C. for 30 minutes, but withstands the action of 50 per cent. glycerol in the ice-chest for several months. It can be grown in tissue culture (Downie and McGaughey 1935), and on the chorio-allantoic membrane of the chick embryo (Paschen 1936). The case mortality of the disease is generally fairly high.

In diagnosis, the chronic foot lesions must be distinguished from those caused by *Actinomyces muris*. This organism can be cultivated directly on serum agar. One of the best methods of demonstrating the presence of the virus in a suspected mouse is to filter a suspension of the liver and spleen through sand and paper pulp, to add 0·5 per cent. phenol, to leave it in the ice-chest till it is bacteriologically sterile, and to inoculate it intraperitoneally into normal mice. If the virus is present, the animals will die within 4-6 days, and macroscopic lesions will be demonstrable in the liver.

Animals that recover from the disease have a substantial immunity. Normal animals can be vaccinated with considerable success by means of a formolized liver suspension (Greenwood and Topley 1936). For a study of the epidemic spread of the disease, see Greenwood and Topley (1936) and pp. 1256–1260.

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CHAPTER 87

FILTRABLE VIRUS DISEASES—*Continued*

C. GROUP CHARACTERIZED BY LESIONS OF THE NERVOUS SYSTEM

ENCEPHALITIS

THERE are a number of diseases, many of them known to be caused by viruses, that affect the central nervous system and give rise to a picture of encephalitis, encephalomyelitis, or myelitis. The classification of these diseases is very difficult, and is generally made on the type of cell attacked. Since this is not a textbook of pathology, we do not wish to discuss the type of lesion produced in these diseases, particularly since there is still considerable disagreement among neuropathologists on this question. Those who wish for a classification along pathological lines may be referred to Rivers (1932) and Hurst (1936).

Fothergill (1941*a*) has suggested a classification based on the degree of neurotropism of the infecting virus: (*a*) caused by strictly neurotropic viruses, i.e. those which attack primarily nerve cells alone, such as the viruses of poliomyelitis, rabies, Borna disease, and pseudorabies; (*b*) caused by pantropic viruses, i.e. those multiplying not only in nerve cells, but in other types of cell, such as the viruses of St. Louis encephalitis, Australian X encephalitis, equine encephalomyelitis, and louping-ill; some of these diseases are insect-borne; (*c*) caused by viscerotropic viruses, such as vaccinia and mumps; these give rise more to a meningitis than to a true encephalitis. In addition, there are secondary types of encephalitis, such as post-vaccinal encephalitis, the causation of which is still unknown, but which are characterized pathologically by perivascular demyelinization.

In the account that follows we shall content ourselves with describing in sequence the more important diseases of man and animals in which the nervous system is affected.

Human Encephalitis

Encephalitis Lethargica.

This is a primary encephalitis, presumably of virus origin. The disease was first described by von Economo (1917) in Vienna, and was recognized in this country the following year. The highest year of incidence in England and Wales was 1924, when 5,039 cases were notified with 1,407 deaths. Since then the incidence has declined gradually, and in 1938 the number of registered deaths had fallen to 650. From post-mortem observations; however, it appears very doubtful whether more than a small proportion of the cases diagnosed as encephalitis lethargica are really examples of this disease. The true incidence is probably far lower than is indicated by the reported figures. *Epidemic hiccup* is regarded as a mild form of the disease (see MacNalty 1937).

Conflicting reports have been published on the ætiology of encephalitis lethargica. Much of the earlier work on the transmission of infection to animals was confused by the occurrence of a spontaneous disease of rabbits—meningo-encephalitis (see p. 1924)—and must be disregarded. Observations by Levaditi and Harvier (1922–23), Perdrau (1925*a*, *b*), da Fano and Perdrau (1927), and Gay and Holden (1933) suggest that the virus of encephalitis lethargica may be related to that of herpes, but this is little more than speculation. Though both McIntosh (1923) and McCartney (1924*b*) have reported the transmission of a fatal encephalitis to rabbits by the direct inoculation of brain suspensions from infected patients, it must be admitted that very little progress has been made in the study of this disease and its causation still remains obscure. (For a short summary of the experimental observations made on this disease the reader is referred to pages 1499–1501 in the 2nd edition of this book.)

St. Louis Encephalitis.—A very remarkable outbreak of encephalitis occurred in St. Louis, Mo., during the late summer of 1933. The epidemic started at the beginning of August and finished about the end of October. Rather over a thousand persons were attacked—an incidence of approximately 1 per 1,000 of the population. The case-mortality rate, which had an average of 20 per cent., showed a striking increase in relation to the age of the patient; thus, under 40 years of age it was less than 10 per cent.; in the 40–50 age group it was 12 per cent., in the 50–60 group 21 per cent., in the 60–70 group 38 per cent., in the 70–80 group 56 per cent., and in the 80–90 group 80 per cent. Over half the fatal cases died in the 1st week. The incubation period was not ascertained with certainty, but appeared to be from 4 to 21 days. Pathologically, the lesions differed in several respects from those in encephalitis lethargica, particularly in the more extensive involvement of the meninges and spinal cord, the more widespread distribution of inflammatory foci through the brain, and the greater frequency of degenerative changes in the nerve cells and of neuronophagia.

Cultures of the blood and spinal fluid were sterile. Attempts to transmit the disease to rabbits proved a failure, but successful results were obtained by Muckenfuss, Armstrong, and McCordock (1933) with *Macacus rhesus* monkeys. Combined intracerebral and intraperitoneal inoculation of human brain suspension was followed in 8–14 days by a steadily rising temperature, drowsiness, intention tremor, muscular weakness and inco-ordination of the extremities, and increased pressure of spinal fluid. Most of the animals were killed, and it is not known whether the disease would have proved fatal spontaneously. The changes in the nervous system observed post mortem were similar to those in the human disease. Only about 40 per cent. of monkeys proved susceptible, and after 5 passages, experiments with these animals were discontinued.

The disease was also shown to be transmissible to *mice* (Webster and Fite 1935*a*). After intracerebral inoculation with human brain suspension these animals develop in 3–4 days a condition characterized by hyperæsthesia, coarse tremors, convulsions, prostration, and death in 4–6 days. The disease can be passed indefinitely through mice. Other animals, with the possible exception of the mule, appear to be refractory. Mice can be immunized against intracerebral infection by subcutaneous or intraperitoneal inoculation with living virus (Webster 1938*b*).

The virus is about 20–30 mμ in size (Webster and Fite 1935*b*, Elford and Perdrau 1935). It has been cultivated by Syverton and Berry (1935) in a medium con-

taining minced living embryonic mouse brain, and by Harrison and Moore (1937) on the chorio-allantoic membrane of the developing chick embryo. It is present in great concentration in infected mouse brain; an intracerebral dose of even 3×10^{-8} gm. often proves fatal to mice. Mouse brain suspension remains virulent for at least 3 months when frozen; it withstands the action of 1 per cent. phenol for 25 days in the ice-chest, but is inactivated by 0·1 per cent. formol at room temperature in 12–18 hours (Brodie 1934*a*). Using the mouse protection test, in which the virus and the serum to be tested are mixed, incubated for 1 hour at 37° C., and inoculated intracerebrally into mice, it was found that the virus was neutralized by about 82 per cent. of sera from convalescent patients, but not by the serum of non-contacts or of patients who had suffered from herpes, encephalitis lethargica, or poliomyelitis (Webster, Fite and Clow 1935, Brodie 1934*a*). Wooley (1934), however, found that about 30 per cent. of sera from persons in different parts of the United States contained neutralizing bodies for the St. Louis virus. Neutralizing antibodies have also been found in Kenya, Congo, and possibly the Sudan, showing that the disease is not confined to the United States (Smithburn and Jacobs 1942). The virus is distinguished from the viruses of herpes and vaccinia by its failure to provoke a response in rabbits, and from those of Rift Valley fever, louping-ill, poliomyelitis, equine encephalitis, and mouse encephalitis by its effects upon monkeys and mice. It is closely related to the virus of Japanese B encephalitis and the West Nile encephalitis virus, but is said by Webster (1938*a*) and Casals (1944)—though Perdrau (1940) disagrees—to be antigenically distinguishable from it. The St. Louis virus is virulent for mice, but only weakly virulent for the monkey: the Japanese virus is virulent for mice, monkeys, and sheep.

The mode of spread of St. Louis encephalitis is not yet clear, but there is strong evidence to suggest insect transmission. Hammon and his colleagues (1942*b*, *c*) have isolated three strains of the St. Louis virus from mosquitoes—*Culex tarsalis* Coquillet—in the Yakima valley and have proved this insect to be capable of transmitting infection under experimental conditions. Diagnosis of the disease is aided by the complement-fixation test (Casals and Palacios 1941); antibodies appear in the blood within two weeks. (For a general account of the disease, see Report 1935.)

Japanese Encephalitis.—SYNONYM: *B encephalitis.*—In Japan several outbreaks of encephalitis have occurred during the past 80 years or so. They have been particularly common during the late summer months, and for this reason the disease is often referred to as "summer encephalitis." A large outbreak occurred in the summer and autumn of 1924, when there were over 7,000 cases with a fatality rate of 60 per cent. Another large epidemic, causing about 5,000 deaths, was witnessed in August and September 1935.

The causative virus was isolated and studied by Kasahara and his colleagues (1936*a*, *b*, 1937*a*, *b*) and by Taniguchi and his colleagues (1936). It is about 20–30 mμ in size (Yaoi *et al.* 1939); it can be cultivated in a medium of the Maitland type containing minced chick embryo brain (Kawakita 1939); it is avirulent to rabbits, but gives rise to a non-suppurative meningo-encephalomyelitis on intracerebral inoculation of mice, monkeys, and young sheep; it is neutralized by the serum of convalescent patients; and it can be distinguished by cross-neutralization tests from the virus of St. Louis encephalitis (Kawamura *et al.* 1936, Kasahara *et al.* 1937*a*, Webster 1938*a*) and from the West Nile virus (Casals

1944). Guinea-pigs are said by the Japanese workers (Kasahara *et al.* 1936*a*) to be susceptible to intratesticular inoculation, but they appear to be resistant to intracerebral inoculation (Webster 1938*a*).

In man the disease can be diagnosed during life by the demonstration of complement-fixing and of neutralizing antibodies in the patient's serum, and at autopsy by inoculation of brain tissue intracerebrally into mice. Histologically, degeneration of the Purkinje cells of the cerebellum is an outstanding lesion. There is some evidence that infection may be transmitted to man by mosquitoes —*Culex tritæniorhynchus* (see Fothergill 1941*a*). Neutralizing bodies to the Japanese B virus have been found in the blood of natives of the Sudan and Congo (Smithburn and Jacobs 1942).

Australian X Encephalitis.—A disease simulating the Japanese encephalitis occurred in Australia in 1917 and again in 1918. It had a high fatality rate—70 per cent. This so-called X disease broke out again in New South Wales in 1922 and 1926. The virus is readily transmissible to monkeys and to sheep, and the lesions to which it gives rise are very similar to those observed in sheep affected with louping-ill (see p. 1925). The X disease may possibly be contracted from sheep (Perdrau 1936).

Russian Spring-Summer Encephalitis.—In 1937 cases of human encephalitis were recognized by Chumakov and Gladkikh (1940) occurring particularly among wood-cutters in thickly forested areas of the Soviet Far East. The disease occurs in the spring and summer months, and is transmitted by the bite of the tick, *Ixodes persulcatus* Schulz. Wild rodents appear to constitute a reservoir of infection in endemic areas. A virus was isolated from the brain of fatal cases, which the Russian workers looked upon as resembling the virus of Japanese B encephalitis. According to Casals and Webster (1944), who studied three strains, and to Casals (1944), it is very similar to, though not identical with, the virus of louping-ill (see p. 1925).

West Nile virus Encephalitis.—Smithburn, Hughes, Burke and Paul (1940) described the isolation of a virus from the blood of an African woman who was suffering only from slight fever, but who subsequently developed neutralizing antibodies in her serum. The agent was designated the West Nile virus, from the district in which it was encountered. Mice and monkeys inoculated intracerebrally developed encephalitis, and degenerative changes were found in the Purkinje cells of the cerebellum recalling those produced by the louping-ill virus. Smithburn (1942) compared the West Nile virus with the viruses of St. Louis and of Japanese B encephalitis. All three were found to be related to each other, but to be distinguishable by cross-neutralization tests. Human sera that neutralized the West Nile virus failed to neutralize either of the other two, but antisera to the St. Louis and Japanese B viruses partly neutralized the West Nile virus. The relationship of this virus to the virus of louping-ill has not yet been determined. It may be noted, however, that the West Nile virus is estimated to be 21–31 mμ in diameter, whereas the louping-ill virus appears to be only 15–20 mμ.

Encephalomyelitis due to Equine virus.—As its name implies, equine encephalomyelitis is essentially a disease of horses (see below). During the late summer and early autumn of 1938 there was a large outbreak in the United States, and human cases of the disease were recognized for the first time. Diagnosis of infection

TABLE 189

PRIMARY ENCEPHALOMYELITIS OF HUMAN BEINGS. (Modified from Webster 1941*b*)

Disease.	Reservoir.	Mode of Transmission.	Size of Virus mμ	Susceptibility of Animals to Intracerebral Inoculation.						Remarks
				Mouse	Monkey.	Sheep.	Rabbit.	Guinea-pig.	Rat.	
Rabies	Animals.	Bite.	100–150	+	+	+	+	+	+	
Poliomyelitis	?	?	8–12	—	+	—	—	—	±	Cotton rat susceptible.
St. Louis encephalitis . .	? animals.	Probably mosquitoes.	20–30	+	±	—	—	—	—	Antigenically related to Japanese B and West Nile viruses.
Japanese B encephalitis .	? animals.	Probably mosquitoes.	20–30	+	+	+	—	—	—	Antigenically related to St. Louis and West Nile viruses.
Australian X encephalitis .	?	?	?	?	+	+	—	—	—	
Russian Spring-Summer encephalitis	Probably rodents.	Probably ticks.	?	+	+	+	—	—	?	Antigenically related to louping-ill virus.
West Nile virus encephalitis	? animals.	?	21–31	+	+	?	—	—	?	Antigenically related to St. Louis and Japanese B viruses.
Louping-ill	Sheep.	Ticks.	15–20	+	+	+	—	—	—	Antigenically related to Russian Spring-Summer virus.
Equine encephalomyelitis .	? animals. or birds.	Probably mosquitoes.	20–35	+	+	±	+	+	+	
Lymphocytic choriomeningitis	Mice.	?	37–60	+	+	?	—	+	±	
" B " myelitis	? monkeys.	?	?	±	+	?	+	+	?	Allied to herpes and pseudorabies viruses.
Encephalitis lethargica . .	?	?	?	—	—	?	?	—	—	Virus not yet isolated.

with the eastern type of virus was made by Fothergill, Dingle, Farber and Connerley (1938) and by Webster and Wright (1938), and with the western type of virus by Howitt (1938*b*). The disease occurred in rural areas, and affected particularly infants and young children, in whom it caused a high mortality. The virus can sometimes be demonstrated in the blood (Howitt 1939) and in the cerebrospinal fluid (Fothergill, Holden and Wyckoff 1939) during life. After death it may be isolated from the brain by intracerebral inoculation of mice. Neutralizing antibodies are often present in the blood serum and the cerebrospinal fluid of convalescent patients (Howitt 1941, Buss and Howitt 1941). Healthy contacts who have been exposed to infection may also show neutralizing antibodies (Mitchell and Pullin 1943). Diagnosis during life may be made by the demonstration of complement-fixing and neutralizing antibodies in the patient's serum (Casals and Palacios 1941, Casals 1941, Howitt 1943). Infection is probably carried by mosquitoes—*Culex tarsalis* Coquillet (Hammon *et al.* 1942*a*, *b*). Besides the disease due to the eastern and western types of virus, laboratory infections have been described with the Venezuelan type (Casals *et al.* 1943).

Bwamba Fever.—A mild non-fatal disease, apparently caused by a virus producing nervous symptoms in mice, was encountered in African natives by Smithburn, Mahaffy and Paul (1941) during a yellow fever survey in the Western Province of Uganda and was named by them Bwamba fever. Clinically, the disease was characterized by a sudden onset with fever, headache, and pain in the lumbar region, mild conjunctival injection and a skin rash. The fever lasted for 2–5 days, the headache and backache for 2 or 3 days longer. Convalescence was rapid and complete. The disease did not appear to be highly contagious. A virus could be demonstrated in the blood by intracerebral inoculation of mice, and could be carried over from one mouse to another by injection of brain tissue. After intracerebral injection symptoms of nervous excitement appeared in about 4 days, and the animals generally died a day later. At post mortem there was a variable degree of hyperæmia and œdema of the brain. Microscopically, widespread degenerative changes were seen in the pyramidal cells of the cortex, together with single or multiple acidophilic intranuclear bodies resembling inclusions. Infection did not spread from mouse to mouse by contact, even though the animals could be infected by intranasal inoculation. Monkeys developed a non-fatal febrile illness when injected intracerebrally. Guinea-pigs and rabbits proved resistant. The virus passed through a Seitz filter. Judged by experiments with gradocol membranes its size was between 113 and 150 mμ. It could be readily preserved by drying from the frozen state, but a saline suspension kept in the ice-chest proved innocuous within 13 days. It was destroyed by exposure to heat at 50° C. for 30 minutes. Antibodies specifically neutralizing the virus were demonstrated in convalescent human and monkey serum. So far as could be ascertained, the 9 strains of virus that were isolated appeared to be antigenically identical.

Semliki Forest Virus.—Under this name Smithburn and Haddow (1944) and Smithburn, Mahaffy and Haddow (1944) have described a virus isolated from a group of *Aëdes abnormalis* mosquitoes caught in the Semliki forest of Western Uganda. It killed mice when inoculated by various routes, but was fatal to guinea-pigs, rabbits and *rhesus* monkeys only on intracerebral inoculation. In the brain it produced lesions resembling those caused by the equine encephalomyelitis viruses. By neutralization tests it was found to differ from other encephalitis viruses, including the West Nile virus, though it was not compared with the virus of Russian spring-summer encephalitis. It also appeared to be smaller in size than any of these viruses. Whether it is pathogenic to human beings is not known, but neutralizing antibodies were found in a proportion of human sera collected in the neighbourhood. It is suggested that the red-tail monkey, *Cercopithecus nictitans*, may form one of the natural reservoirs of the virus.

Ascending Myelitis. " B " virus and herpes virus.—Sabin and Wright (1934) recorded a human case of acute ascending myelitis, associated with focal necroses in the viscera, following the bite of an apparently normal *Macacus rhesus* monkey. By the inoculation of rabbits a strongly neurotropic filtrable virus was demonstrated in the patient's brain, cord and spleen. In monkeys the neurotropic tendency was not so evident. Intravenous inoculation of these animals was followed by a generalized disease not unlike that caused by vaccinia, and accompanied by a rash on the face, buccal mucosa, and conjunctiva (Sabin 1934). Besides rabbits and monkeys, guinea-pigs are also susceptible to experimental inoculation. The virus, which is usually referred to as the " B " virus, can be cultivated on the chorio-allantoic membrane of the developing chick embryo (Burnet, Lush and Jackson 1939*a*). Infection appears to be endemic in monkeys, in which neutralizing antibodies can often be demonstrated; morbidity and mortality in these animals, however, are very low.

Primary myelitis may follow the intravenous inoculation of *herpes* virus into rabbits (Koppisch 1935, Doerr and Hallauer 1936). We shall subsequently have occasion to refer to cases of acute ascending myelitis of the Landry type caused by the virus of rabies.

Burnet, Lush and Jackson (1939*b*) have drawn attention to the similarity between the B virus, the herpes virus, and the pseudorabies virus. All produce intranuclear inclusion bodies; all are predominantly but not strictly neurotropic; all may appear in the blood after inoculation into a distal part; but they invade the central nervous system, not like louping-ill, equine encephalomyelitis and some other viruses by way of the blood, olfactory mucosa, and olfactory bulbs, but by the peripheral nerves; all tend to produce death in experimentally inoculated animals at a time when little virus is present in the brain; and all are comparatively innocuous to hosts in which they naturally cause endemic infection, but are highly fatal to other animals.

Secondary Encephalitis.—We have already (p. 1894) drawn attention to the encephalitis that occasionally follows vaccination against smallpox, and have discussed its ætiology. Similar cases of encephalitis sometimes occur during convalescence from other diseases such as measles, varicella, mumps, yellow fever, and influenza. The most striking characteristic of all these cases is the occurrence of perivascular demyelination—a lesion which is not usually met with in primary encephalitis. Whether the production of secondary encephalitis is due to the virus causing the primary disease, or to a different virus lying latent in the tissues, or is a purely degenerative condition not determined by a virus at all, remains a subject for debate (see McIntosh 1928, McIntosh and Scarff 1928, Greenfield 1930, Rivers 1932, Ledingham 1935, Hurst 1936).

For a description of the clinical and pathological manifestations of the various types of encephalitis in human beings, the reader is referred to the monograph by Neal (1942).

Encephalitis in Animals

Borna Disease. Endemic Encephalomyelitis of Horses, Cattle and Sheep.

Borna disease is the name given to an infectious encephalomyelitis of horses. The name " Borna " is derived from the locality in Saxony where a severe epizootic occurred during the years 1894–96. The disease is widely distributed, and may occur in epidemic form with a mortality sometimes reaching 90 per cent. A similar

encephalomyelitis is likewise observed in cattle and sheep. Histologically, lesions are found in the central nervous system and in the peripheral nerves, and characteristic inclusions (Joest-Degen intranuclear corpuscles) are observed in the large ganglion cells of Ammon's horn. The disease is due to a filtrable virus, which appears to be the same in all three species of animal. According to Elford and Galloway (1933*b*) it is about 85–125 mμ in diameter. The virus is resistant to glycerol, but sensitive to desiccation, ultra-violet light, and heat. Inoculated intracerebrally into rabbits it gives rise after an incubation period of 15 to 50 days to a disease characterized by cerebral symptoms and proving fatal in 5 to 17 days. The virus produces lesions in the central nervous system, and makes its way down the peripheral nerves, in which it sets up an interstitial neuritis. Monkeys, guinea-pigs, and rats are also susceptible. Rabbits may be immunized against the disease by the inoculation of suitably attenuated virus into the brain; sometimes immunity is produced by multiple inoculations subcutaneously of large quantities of formolized vaccine. There is some evidence that the Borna virus is related to the virus of poliomyelitis. (For an excellent review of the disease, together with an account of original work, see Nicolau and Galloway 1928.)

Encephalomyelitis of Horses.—This is an endemic disease of the United States and Canada, which has, however, been recognized in parts of South America. It resembles the Borna disease of Europe, differing from it in the histological character of the lesions and in the type of the causative virus (Hurst 1934, Howitt 1935). In the United States it has been observed mainly along the Atlantic seaboard and in California. The disease occurs in the late summer and autumn. The eastern type is seen in salt marshes, the western type in river valleys. There is strong evidence to suggest that the disease is spread by insects (for references see Fothergill 1941*b*). Kelser (1933) showed experimentally that infection could be transmitted by the mosquito, *Aedes ægypti*. More recently the western type of virus has been demonstrated under natural conditions in another mosquito, *Culex tarsalis* Coquillet (Hammon *et al.* 1942*b*, *c*). In 1937 and 1938 there was a very large outbreak of the disease, several thousand horses dying. At the same time, a considerable mortality was observed among pigeons (Fothergill and Dingle 1938) and pheasants (Tyzzer, Sellards and Bennett 1938); from both of these birds the eastern type of virus was isolated. A field study by Hammon and his colleagues (1942*a*) in the Yakima valley revealed the presence of neutralizing bodies for the western type of virus in the sera of about 50 per cent. of domestic birds and 35 per cent. of domestic mammals studied, and of about 20 per cent. of wild birds and 8 per cent. of wild mammals. It would appear therefore that the disease is endemic in animals and birds, and that, as suggested by Tenbroeck, Hurst and Traub (1935), the horse is not the primary reservoir of infection. It is of interest to inquire how an epidemic disease of the central nervous system can be spread by a blood-sucking insect. The answer appears to be that, after gaining access to the horse, the virus invades the blood stream and multiplies in the viscera. After this first pyrexial stage of the disease, the virus disappears from the blood stream and reaches the central nervous system—apparently by way of the cranial nerves. The horse is therefore infective for the mosquito during only a brief period. This is an additional reason for believing that the virus has its habitat in other animals.

The cause of the western type of the disease was found by Meyer, Haring and Howitt (1931) to be a filtrable virus. A similar virus, differing from it immuno-

logically was later shown by Ten Broeck and Merrill (1933), Howitt (1935), Records and Vawter 1935) and Shahan and Giltner (1935) to be responsible for the eastern type. Both viruses are estimated to be about 20–35 mμ in diameter (Bauer *et al.* 1935, Tang *et al.* 1937), though Sharp and his colleagues (1943) give a figure of about 50 mμ. They can be grown readily in tissue culture and on the chorio-allantoic membrane of the developing chick embryo. They remain viable in 50 per cent. glycerol in the ice-chest for a long time, but are readily destroyed by formalin. The eastern type is said to be more pathogenic than the western for guinea-pigs and rabbits, but both have a wide range of pathogenicity. The Argentine type of virus studied by Howitt (1935) appears to be identical with the western type, but the Venezuelan type, described by Beck and Wyckoff (1938) and Kubes and Ríos (1939), is immunologically distinct. Howitt (1938*a*) likewise found a Russian strain, labelled Moscow 2, to be immunologically distinct from the other viruses. The Russian virus, however, differs in important respects from the viruses of equine encephalomyelitis met with in the western hemisphere. It is much larger, having a diameter of 85–130 mμ (Lazarus and Howitt 1937), and has not so far been grown in tissue culture or in the chick embryo. In both these respects it resembles the virus of Borna disease, from which it seems to be distinguished mainly by its antigenic structure and its greater invasive power for animals (Howitt 1938*a*). Since morphology must be one of the primary characters on which classification is based, it would seem wise to regard the Russian virus as allied to the Borna rather than to the American group of viruses.

In protection against equine encephalomyelitis inoculation with a formolized chick embryo vaccine, using the technique of cultivation described by Higbie and Howitt (1935), appears to be hopeful (see Eichhorn and Wyckoff 1938, Fothergill 1941*b*).

Encephalitis of Foxes.—This disease is endemic in certain parts of the United States, and at times assumes epidemic proportions. It is caused by a filtrable virus which invades the blood, probably through the respiratory mucosa, and may be demonstrated in various tissues. Clinically the disease is characterized by anorexia, hyperexcitability or lethargy, convulsions, paralysis, and not infrequently by death. Pathologically, hæmorrhages in the brain and cord and in the viscera constitute the most striking feature of the disease; perivascular infiltration with round cells is common in the cerebral nervous system, and the meninges are usually involved. Characteristic intranuclear inclusion bodies are found in the ependymal and endothelial cells of the central nervous system. The disease can be reproduced by intramuscular, intranasal, or intracranial inoculation of young foxes with homogenized brain and spinal cord. Unlike the natural disease, which seldom has a case mortality exceeding 20 per cent., the experimental disease is fatal in about 70 per cent. of cases. Infection can also be transmitted to dogs, though with some irregularity. (For an account of the disease see Green *et al.* 1930, 1931, 1933, 1934, 1936, Green 1931, 1936, Green and Shillinger 1934.)

Encephalomyelitis of Mice.—This disease has been described by Theiler (1937) in the United States, Gildemeister and Ahlfeld (1938) in Germany, and Iguchi (1939) in Japan. It is characterized by flaccid paralysis of the limbs and sometimes convulsions. Infection can be transmitted by intracerebral, intranasal and intraperitoneal inoculation. After intracerebral inoculation of mice there is an incubation period of 5–30 days. Flaccid paralysis then develops, usually affecting the fore limbs first, but being more severe in the hind limbs. Death

usually follows 5–10 days after the onset of paralysis, but some animals survive and remain carriers for a time. Paralysed and carrier mice are resistant to a second intracerebral inoculation. There is evidence that immunity depends on the presence of neutralizing antibodies in the blood serum. Histologically, perivascular cellular infiltration is found throughout the central nervous system; there is acute necrosis of the ganglion cells, particularly in the anterior horn, followed by neuronophagia. No inclusion bodies appear to have been demonstrated yet.

The virus is one of the smallest known—9 to 13 mμ—resembling in this respect the virus of poliomyelitis. It is readily destroyed at temperatures above 50° C., and by 20 per cent. alcohol, but is resistant to ether and to 50 per cent. glycerol (Theiler and Gard 1940). It is non-pathogenic for *rhesus* monkeys and for rabbits. Infection seems to be widespread in colonies of mice, though only a small proportion of animals develop the characteristic disease. According to Gard (1944), infection may perhaps be activated by organisms of the *Salmonella* and other groups that give rise to enteritis. By the time they are 4 weeks old nearly every mouse in a colony in which the disease is endemic appears to be infected. The virus is present in the mucosa of the stomach and intestine, the mesenteric lymph nodes and the fæces of young mice, but can no longer be found after the animals reach the age of 6 months. By this time immunity has been acquired (see Olitsky 1939*b*, 1940, Theiler and Gard 1940).

Infectious Encephalomyelitis of Chickens.—This disease, which is sometimes referred to under the name of "epidemic tremor of chickens," was reported by Jones (1934) in New England. It is characterized by tremor, most noticeable in the head and neck, and progressive ataxia, either or both of which may be present in a single bird. Young chickens are mainly attacked, the usual age of onset being about 3 weeks. In commercial flocks the morbidity ranges from 5 to 50 per cent. In severe outbreaks about half the chicks may die; death is due to inanition caused by the progressive ataxia. Histologically, focal collections of glia cells, perivascular infiltration, and degeneration of nerve cells, especially of the Purkinje cells in the cerebellum, are found in the central nervous system. No inclusion bodies have yet been demonstrated. The disease can be reproduced in young chicks by intracerebral inoculation of brain and cord from affected birds. The incubation period of the experimental disease is usually 2–4 weeks; death follows about a week after the onset of symptoms (van Roekel *et al.* 1938). The infective agent is probably fairly large, since it can pass through a Berkefeld N but not a W filter. According to Olitsky (1939*a*), birds that recover from the disease are immune, and neutralizing antibodies can be found in their blood serum.

Meningo-encephalitis of Rabbits.—Though this is not a virus disease, it may be conveniently considered here, since it is of considerable laboratory importance and has in the past caused much confusion, especially in work on encephalitis lethargica. In 1922 Oliver described a spontaneous chronic meningo-encephalitis affecting 20 per cent. of her normal stock rabbits. Similar lesions in a few animals had been observed a few years previously by Bull (1917), while working on streptococci isolated from poliomyelitis. In this country the disease was described independently by Twort (1922) and Twort and Archer (1923), who likewise met with it while investigating a different problem. McCartney (1924*a*) found that 55 per cent. of the stock rabbits at the Rockefeller Institute in New York were infected.

The disease may occur in epizootics; symptoms are usually absent. Histologically, in the brain there are infiltrations with lymphocytes and plasma cells, occurring around the vessels, in the meninges, and in the cerebral substance itself, where the cells may form local aggregations or be widely diffused. In 15 per cent. of the rabbits there are, in addition, focal areas of necrosis, consisting of a necrotic centre surrounded by epithelioid cells, around which is a layer of mononuclear cells. In the centre of these necrotic lesions, there may be rounded or oval cyst-like bodies, containing numbers of small spherical, spindle- or pear-shaped particles, similar to those described by Levaditi under the name of *Encephalitozoon cuniculi*. These bodies generally consist of a thin structureless membrane, and a protoplasmic mass with regularly distributed chromatin particles; on careful examination, it is found that the protoplasmic mass really consists of small spindle- or pear-shaped portions, each of which is provided with a particle of chromatin. This parasite appears to be a microsporidium belonging to the *Sporozoa*; it has been found both in the brain and in the kidney lesions that occur in the disease. Under natural conditions this spontaneous meningo-encephalitis is spread by cage infection; experimentally it can be transmitted by inoculation of nervous substance and the sedimented urine of infected animals. The parasite was described by Wright and Craighead in 1922, before Oliver had drawn attention to the spontaneous disease to which it gives rise. Cowdry and Nicholson (1924) have found a similar parasite in spontaneous encephalitis of mice (see also Pette 1928).

A similar disease has been reported by Markham (1937) in guinea-pigs.

Louping-ill.—This disease is an encephalomyelitis of sheep characterized by cerebellar ataxia. The word " louping " is derived from an old Norse word meaning " leap," and refers to the peculiar gait of the animals which spring up and down when moving forwards. The disease seems to be limited to the border counties of England and Scotland and to the Western Highlands. It occurs in early spring, and is commonest on farms where there is rough hill grazing (Pool 1934). It has been studied mainly by workers in Scotland, who have shown (1) that the disease is caused by a virus which invades the blood stream and sometimes, but by no means always, affects the nervous system; (2) that infection is carried by the tick *Ixodes ricinus* L., which is habitually present on louping-ill pastures; (3) that infection can be transmitted by intracerebral inoculation to sheep, pigs, and mice; (4) that the disease is often abortive; (5) that recovery from the overt, abortive, or latent disease is followed by a substantial degree of immunity; (6) that some protection can be afforded by the use of formolized vaccine inoculated subcutaneously; (7) that destruction of the Purkinje cells in the cerebellum is one of the characteristic pathological lesions of the disease; and (8) that an entirely different disease —tick-borne fever—suspected to be of rickettsial origin, sometimes co-existed with louping-ill and increased its severity (see Pool *et al.* 1930, Alston and Gibson 1931, Brownlee and Wilson 1932, Gordon *et al.* 1932, MacLeod and Gordon 1932, Gordon 1934). The disease may also be transmitted to monkeys (Hurst 1931*a*) and to field voles (Findlay and Elton 1933). Both mice and monkeys can be infected by intranasal as well as by intracerebral inoculation (Elford and Galloway 1933*a*, Galloway and Perdrau 1935). The virus can apparently reach the central nervous system from the nose without entering the blood stream. Rivers and Schwentker (1934) appear to have observed the disease in laboratory workers. Clinically the picture was that of an influenza accompanied by encephalitis. Recovery occurred, and neutralizing bodies were demonstrated in the patients' sera. The virus, however, was not isolated. According to Elford and Galloway (1933*a*), the causative virus is about 15–20 mμ in diameter. Even after 40 mouse passages, it still remained infective for sheep.

POLIOMYELITIS

Poliomyelitis is principally a disease of young children, which occurs sporadically and epidemically. In the northern hemisphere epidemics are commonest in the late summer and early autumn months, the maximum incidence of the disease being reached in August or September. During the present century the United States of America has experienced three major outbreaks—in 1916, 1931 and 1943. The disease resembles cerebrospinal meningitis in attacking only a small proportion of the population; though, as in meningitis, there is reason to believe that infection of a considerable proportion of the population occurs without giving rise to clinical evidence of disease. The case mortality of the declared disease is usually about 10–15 per cent., but it may reach a figure of even 40 per cent. The proportion of cases that show residual paralysis is difficult to estimate, because there is an increasing tendency among clinicians to diagnose the disease in the pre-paralytic stage. In this way a considerable number of abortive cases are included that do not develop paralysis at all. Probably not more than about 15–40 per cent. of the total cases show residual paralysis, and in many of these partial or even complete restoration of function ultimately occurs. Males are affected more often than females.

In the past poliomyelitis has been largely a disease of infancy and early childhood, but of late years the older age groups have been progressively more and more affected (see Burnet 1940). Thus, in the Danish outbreak of 1934 no fewer than 33·2 per cent. of the cases were in persons aged 15 years or more (Jensen 1935). The incubation period is not known with certainty, but is believed to be about 7–14 days. Multiple cases in families are uncommon, and when they do occur are usually more or less simultaneous (Aycock 1941). The disease has been increasing in prevalence during the past 40 years or so; and, though this country has suffered but little, severe epidemics have occurred in other parts of Europe and in North America. The incidence diminishes as the tropics are approached.

The distribution of the cases in an epidemic is irregular. Wickman (1907), who investigated the big Swedish epidemic, found that the disease spread along the lines of communication, railways, roads, and so on. He brought evidence to show that infection was spread by carriers and by cases. Since it has been found that perfectly healthy persons may carry infection, and that patients may be infective in the incubation period, as well as for a week or two after the commencement of the disease, it is obvious that the prevention of infection by the isolation of cases or quarantining of contacts is not likely to be successful. The disease rarely becomes epidemic in schools or institutions, but it may attack a rural community in a characteristically epidemic form. Thus in the little village of Stokes Rivers, in Devonshire, 45 persons were attacked out of a total population of 119.

Mode and Route of Infection.—The mode of infection is still obscure. There is a mass of evidence pointing in different directions—one to the respiratory, the other to the alimentary route.

Largely under the influence of Flexner, who studied the experimental disease in *rhesus* monkeys, the prevailing belief for many years was that infection was carried by naso-pharyngeal droplets; that the virus invaded the terminal processes of the olfactory nerves—the so-called olfactory hairs; and that it spread along the axis cylinders to the brain, and thence to the anterior horn cells of the cord, where the chief lesions are produced.

The evidence in favour of the intraneural passage of the virus from the periphery to the centre is strong (Fairbrother and Hurst 1930, Hurst 1930, 1936, Faber and Gebhardt 1933, Leake 1935, Bodian and Howe 1941*a*, *b*); but the evidence in favour of nasal infection in human beings is much less convincing. It is true that the virus has been demonstrated occasionally in the nasopharynx of patients suffering from the typical and from the abortive form of the disease, and occasionally of healthy carriers; but observations on human beings who have died of the disease suggest that the olfactory bulbs are infected in only about 10 per cent. of cases (Burnet 1940, Kessel *et al.* 1941), or even less (see Howe and Bodian 1941*b*). On the other hand, the virus is frequently present in the pharyngeal tissues, the intestinal contents, and the stools, particularly of children (see Kling, Pettersson and Wernstedt 1912, Sawyer 1915, Trask, Vignec and Paul 1938, Trask, Paul and Vignec 1940, Trask and Paul 1941, Sabin and Ward 1941, Kessel *et al.* 1941, Langmuir 1942) It appears to be far commoner in the intestinal contents than in nasal washings, suggesting that multiplication may occur in the alimentary tract itself. Kessel and his colleagues (1941), for example, isolated the virus from the stools of 20 per cent. of patients, but not once from 139 nasal washings. Sabin and Ward (1941), who record similar findings, failed also to show the presence of the virus in the saliva. The virus can be demonstrated in the fæces, not only of patients, but of contacts during an outbreak. Thus, Langmuir (1942) found it in the fæces of 4 out of 5 cases and of 20 out of 27 intimate contacts. The studies of Pearson and his colleagues (Pearson and Rendtorff 1945, Pearson *et al.* 1945) seem to show that the virus is restricted very largely to cases and close contacts. Thus, during an epidemic at Forth Worth, Texas, in 1943 the virus was demonstrated in the stools in 6 out of 8 households containing 27 familial contacts, in 8 out of 45 households containing 80 non-familial contacts, and in only 2 out of 127 households containing 374 non-contacts. Similarly, Kessel and Moore (1945), who examined tonsils and stools from non-contacts during an inter-epidemic period, isolated the virus from only 5 out of 136 persons studied. It is present in the spinal cord in fatal cases, but is never found in the blood, the cerebrospinal fluid, or the urine.

The interpretation of these findings is difficult. We must try to relate them to the epidemiological picture. The evidence pointing to the alimentary route of infection is briefly that the disease occurs in the summer and early autumn months; that the virus appears to be commoner in the alimentary tract than in the nose; that it may persist for a long time in convalescent and healthy carriers (see Trask and Paul 1941); that it is often present in sewage (Paul, Trask and Gard 1940, Paul and Trask 1941, 1942); and that it has been isolated from flies (Trask, Paul and Melnick 1943). In addition, *cynomolgus*, but not *rhesus*, monkeys can be infected by feeding; outbreaks of milk-borne infection have been described (see Aycock 1941); and several cases of the disease have been reported after tonsillectomy, though nearly always of the uncommon bulbar form. On the other hand, the disease does not behave epidemiologically like a water-borne disease (see Maxcy 1943); and milk-borne infections are not uncommon in diseases, such as scarlet fever and diphtheria, that are normally transmitted by droplets. The natural spread of infection in an epidemic is far more like that of a respiratory infection, such as cerebrospinal meningitis, though the seasonal incidence is different. Burnet (1940) points out that in the Victorian outbreak of 1937–38 there was clear evidence of the spread of infection by patients—especially abortive cases—and that the only likely mode of transference was by droplet infection. He showed that *cynomolgus* monkeys could be infected by simple swabbing of the pharyngeal mucosa with infected material (see also Faber, Silverberg and Dong 1944). He concludes that under natural epidemic conditions infection usually occurs by inhalation or ingestion of contaminated saliva from an infected child, and that

the virus gains access to the brain by the cranial nerves supplying the pharyngeal mucosa. Sabin (1941), influenced more by the frequent presence of the virus in the fæces and by his failure to find it in the saliva, believes that the virus multiplies in the walls of the pharynx and small gut and invades the medulla or spinal cord by the cranial nerves or by the sympathetic and parasympathetic nervous systems. Bedson (1943) likewise favours the alimentary route of infection.

It is impossible to come to any final conclusion on the usual mode of infection of human beings. On the whole, the evidence points towards the tonsillo-pharyngeal mucosa as being one of the commonest sites of invasion, and to infection being generally air-borne rather than food-borne; but whether the infective air-borne material consists of infected droplets expelled from the nose and mouth or of dust contaminated by fæces is still open to question. Insect-borne infection, which has been suggested, is improbable, mainly because the virus has never been demonstrated in the blood stream, though flies might, of course, become contaminated from fæces and sewage.

Transmission to Animals.—Poliomyelitis is readily transmissible to monkeys. Other animals, with the exception of the cotton rat and the white mouse, which may be infected by specially adapted strains, are insusceptible.

Landsteiner and Popper (1909) were the first to transmit the disease to monkeys; they injected the spinal cord from two fatal cases intraperitoneally into two monkeys. One animal became paralysed in the lower extremities, and died on the 6th day after inoculation; the other was killed on the 19th day. At necropsy, lesions of the spinal cord, similar to those in man, were found in both monkeys. Later in the same year, Flexner and Lewis (1910), using cords from two human cases, succeeded in infecting monkeys (*Macacus rhesus*) by intracerebral, subcutaneous, intraperitoneal, intravenous, intrathecal and intraneural inoculation; intratracheal inoculation and feeding proved unsuccessful. They were able, in addition, to carry over the disease from monkey to monkey by subdural injection of cord suspensions. Flexner and Lewis showed that the disease could be produced, not only by crude cord suspensions, but also by Berkefeld filtrates of these suspensions, and by filtrates of nasopharyngeal washings. Intranasal instillation is also successful (see Flexner 1935), and with some strains intracutaneous inoculation (Trask and Paul 1936).

The disease in *monkeys* is similar to that in human beings. After intracerebral inoculation, there is an incubation period of about 6 to 10 days. During the prodromal period the monkey becomes excitable, the fur is ruffled, there is a high-pitched staccato cry, and the animal easily becomes tired. Within a few hours ataxia develops, accompanied by tremor of the arms, legs or head. The ataxia rapidly merges into a flaccid paralysis, which extends to practically all the muscles of the body except those of the tail, jaws and diaphragm. The temperature drops to subnormal, the animal becomes weaker, and dies within 3 days after becoming prostrate (Amoss 1928). At necropsy, the lesions are similar to those in human beings, and may be briefly summarized as follows: (1) The lesions are chiefly in the grey matter of the cord; the brain is only slightly affected. (2) There is a marked perivascular sheath infiltration, and a more diffuse infiltration of the grey matter of the cord; the cells consist chiefly of lymphocytes, with a fair proportion of polymorphs and a small number of large cells with clear nuclei; plasma cells and lymphoblasts are rare. (3) There is marked degeneration of the ganglion cells of the grey matter; all stages of cellular degeneration, from a slight chromatolysis to complete neuronophagia can be seen. (4) Hæmorrhages are usually present in the grey matter of the cord (McIntosh

and Scarff 1928, Hurst 1929). Characteristic intranuclear inclusion bodies have been described in the nerve cells of fatal human cases, though in monkeys they are less easy to demonstrate owing to the very rapid necrosis that these cells undergo (Hurst 1931*b*).

Though Flexner, using *M. rhesus* monkeys, was unable to set up the disease by feeding, Kling, Levaditi and Hornus (1934) showed that *M. cynomolgus* monkeys were susceptible. The results of these workers were confirmed by Lépine and Sédallian (1939) and Vignec, Paul and Trask (1939). Chimpanzees can likewise be infected by the mouth, even after bilateral section of the olfactory tract (Howe and Bodian 1941*a*). Aisenberg and Grubb (1943) record the successful production of poliomyelitis in *rhesus* monkeys by instillation of infective material into the pulp canal of three anterior teeth. The cavities were immediately sealed to prevent escape of the virus. After 8 days all four legs were paralysed. At post mortem, lesions were found in the Gasserian ganglions, suggestive of a direct nervous spread to the brain.

Armstrong (1939) showed that the Lansing strain of poliomyelitis virus, which had been passed through monkeys, could be transmitted by intracerebral inoculation to *cotton rats* (*Sigmodon hispidus hispidus*); and Jungeblut and Sanders (1940) showed that, after becoming adapted to the cotton rat, the strain could be passed on to *white mice*. The incubation period in cotton rats is 3–8 days, in white mice 2–10 days. Paralysis of one or more legs is first observed and death occurs from respiratory failure in 24–48 hours. Recovery is uncommon (Armstrong 1941).

Properties of the Virus.—Flexner and Noguchi (1913) described the cultivation of minute globoid bodies, 0·15–0·3 μ in diameter, in an ascitic fluid rabbit kidney medium, and brought evidence to show that these bodies constituted the causative agent of the disease. The balance of evidence, however, is definitely against this conclusion (see Fairbrother 1929). More recent work has shown that the virus is probably much smaller—12–17 mμ according to Theiler and Bauer (1934), 8–12 mμ (Elford *et al.* 1935). It has proved very difficult to grow in culture. Sabin and Olitsky (1936), however, appear to have been successful in cultivating it in the presence of nervous tissue removed from human embryos. It is very susceptible to heat, being destroyed in 30 minutes by exposure to a temperature of 45°–50° C. It is killed rapidly by ultra-violet light (Jungeblut 1937). On the other hand, it is resistant to freezing, drying, X-rays, sonic vibration, and numerous protoplasmic poisons (see Jungeblut 1937). It survives in water for a month in the light and 3–4 months in the dark. It remains active in fæces at ice-box temperature for several months, but dies out in a few days in sewage. It withstands 1 per cent. phenol and 15 per cent. ether for several months at 0° C. (Paul, Trask and Gard 1940, Paul and Trask 1942). In 50 per cent. glycerol it can be preserved for 4 to 6 years (Amoss 1928). Little is yet known about its antigenic structure, but cross-neutralization tests carried out with the sera of convalescent human beings and monkeys show that several immunologically distinct types of virus exist. The serum of monkeys convalescent from infection with one type may fail to protect against infection with another, and convalescent monkeys immune to re-inoculation with the homologous type can be re-infected with a heterologous type (see Burnet and Macnamara 1931, Southby and McKie 1933, Paul and Trask 1933, Trask *et al.* 1937, Burnet and Keogh 1938, Kessel *et al.* 1939, Stimpert and Kessel 1939).

Immunity.—In man second attacks of poliomyelitis are rare, but they do occur. Monkeys can often be re-infected successfully, with either the homologous or a heterologous strain (Flexner 1937). Active immunization of monkeys against intracerebral inoculation is possible by repeated intracutaneous inoculation with

living virus, but complete protection is not easy to obtain (Aycock and Kagan 1927, Stewart and Rhoads 1929). The serum of convalescent patients and monkeys, and of actively immunized monkeys, frequently but not always, contains substances capable of inactivating the virus *in vitro*. Observations have shown that neutralizing bodies are often present in the serum of persons who have never suffered from the clinical disease or been in known contact with infection. The test for these bodies is usually made by mixing the serum with a virus suspension, incubating at 37° C. for 2 hours and keeping in the ice-chest overnight, and injecting the mixture intracerebrally into *rhesus* monkeys (Aycock and Kramer 1930). Excluding new-born infants, whose blood often contains temporary antibodies, it is found that neutralizing bodies in the serum increase in frequency with age. In urban districts approximately 75 per cent. of children contain them by the age of 15 years. Most workers have concluded that these bodies represent specific antibodies resulting from latent infection and immunization. This view necessitates the belief that poliomyelitis infection is much more widespread than has hitherto been believed. The difficulty of accepting such a conclusion has led various workers, notably Jungeblut and his colleagues (Jungeblut 1933, Jungeblut and Engle 1933, 1934), to suppose that these antibodies are developed as part of the normal physiological maturation of the individual and do not necessarily denote previous exposure to infection. More assumptions appear to be entailed in this view than in supposing that active immunization is responsible. We do not wish, however, to discuss this question here, since reference has already been made to it in Chapter 49. Until we are in a better position to interpret the significance of the presence of neutralizing bodies in the serum, we shall be wise to refrain from drawing too far-reaching conclusions on the epidemiology of the disease.

It may be mentioned that there are no satisfactory serological or skin tests to help in the *diagnosis* of the disease. Cell changes in the cerebrospinal fluid are often detectable, but the virus itself can never be demonstrated in the fluid.

Prophylaxis, Vaccination and Serum Treatment.—If our views on the frequency of latent infection are correct, it is clear that the detection and removal of carriers are as little likely to be successful in preventing poliomyelitis as they are in preventing cerebrospinal meningitis. There is, however, evidence to suggest that infection is restricted mainly to cases and close contacts, so that control measures over such persons may be worth taking (Pearson and Rendtorff 1945, Pearson *et al.* 1945). General protective measures must follow those common to all droplet-borne respiratory infections. During an outbreak overcrowding should be avoided, ventilation should be increased, and children should spend as much time in the open air as possible. Operations on the nose and throat should be postponed, unless urgent. Cases and close contacts should be segregated for at least 3 weeks. Children in an infected house should be kept away from school for the same length of time. The advantage of closing residential schools is doubtful; and the balance of opinion is against it.

Great hopes were raised by the work of Armstrong and Harrison (1935, 1936) on the *chemoprophylaxis* of the disease. These workers brought evidence to suggest that the application to the nasal mucosa of a 2–4 per cent. solution of alum, or better of a 0·32–0·64 per cent. solution of picric acid, was capable of increasing the local resistance of monkeys to intranasal infection with the virus. Schultz and Gebhardt (1937) found that a 0·5 per cent. solution of zinc sulphate was even more effective. Field trials, however, on human beings in the United States and Canada have been disappointing (see Armstrong 1937,

Tisdall *et al.* 1937, Schultz and Gebhardt 1942). Not only have these agents no apparent prophylactic effect, but they may do considerable local damage by causing extensive coagulation necrosis of the olfactory epithelium. Moreover, since there is serious doubt as to whether infection in man occurs by the nose, the rationale of this form of prophylaxis rests on very slender grounds. The method has now been generally abandoned.

Attempts to *vaccinate* monkeys, and later children, against the disease have been numerous of late years, though it seems clear that mass immunization would be practicable only under quite exceptional conditions. Kolmer and his colleagues (Kolmer and Rule 1934*a*, *b*, Kolmer 1935, Kolmer *et al.* 1935) have used a 4 per cent. suspension of infected monkey cord in 1 per cent. sodium ricinoleate. The virus, though living, is said to be attenuated. Brodie (1934*b*) and Brodie and Park (1935), on the other hand, recommend a vaccine in which the virus has been inactivated by exposure to 0·1 per cent. formol at 37° C. for 8–12 hours. Both of these vaccines have been tried on a small scale in the United States. The demonstration, however, by Olitsky and Cox (1936) that Kolmer's vaccine may occasionally give rise to poliomyelitis in monkeys, and the even more serious development of poliomyelitis in children, occurring apparently as the result of prophylactic vaccination (Leake 1935), have led to the discontinuance of these vaccines.

As has already been pointed out, the blood *serum* of convalescent human patients often contains immune bodies capable of neutralizing the virus. During an epidemic, serum from such convalescent patients may be injected prophylactically into young children who have been exposed to infection, in a dose of 10 ml. subcutaneously, the injection being repeated in a month if the epidemic persists. Serum from human convalescents may likewise be used for treatment. According to Aycock and Luther (1928), it should be given in the pre-paralytic stage of the disease. By giving intravenous and intraspinal injections of serum during this stage, they obtained evidence that the incidence and severity of the subsequent paralysis was decreased in comparison with untreated patients (Aycock *et al.* 1929). Further experience, however, of serum treatment, even in the pre-paralytic stage of the disease, has yielded disappointing results. Park (1932) collected figures referring to the treatment of 439 patients in the pre-paralytic stage of the disease with serum. The serum was given subcutaneously, intramuscularly, intravenously, intraspinally, or by two or more of these routes in combination. The total dosage amounted to 25–100 ml. The results, which are given in Table 190, failed to

TABLE 190

SHOWING THE CONDITION OF TREATED AND UNTREATED CASES OF POLIOMYELITIS AT THE END OF THREE WEEKS (After Park 1932).

	No. of Cases.	Died. Per cent.	Paralysis. Per cent.	Weakness. Per cent.	No Paralysis or Weakness. Per cent.
Treated	519	3·8	19·6	7·7	68·8
Controls	408	0·9	11·0	14·2	73·7

show any advantage, as regards the development of paralysis, of the serum-treated over the control patients. Somewhat similar results are recorded by Fischer (1934), who compared 477 serum-treated patients in the pre-paralytic stage with 102 controls. Both as regards case mortality and the degree of paralysis the control group showed some slight, though probably insignificant, advantage over

the serum-treated patients (see also Schultz and Gebhardt 1935, Jensen 1935, Eagles *et al.* 1937, Aycock 1941).

On general grounds it seems very improbable that serum would exercise any beneficial effect. The virus is never found in the blood, and circulating antibodies are incapable of reaching it in its intraneural situation (see Perdrau 1938, Faber 1941). Mixed *in vitro* with the virus, neutralizing antibodies are effective, but not if injected separately into the infected animal (Armstrong 1941). These considerations are supported by Burnet's (1940) finding that the neutralizing antibody level may be unaffected by passage of a patient through a clinical attack of the disease, and that persons who develop the disease often have relatively large amounts of circulating antibody at the time of their illness; and by Howe and Bodian's (1945) observation that the administration of homologous hyperimmune serum to chimpanzees before alimentary infection has apparently no effect, as judged by comparison with control animals, on the development or severity of the disease. The apparently beneficial effect of serum that has occasionally been reported in field trials would appear to be due in large part, if not wholly, to the inclusion in the treated group of mild, non-paralytic cases that are not ordinarily reported.

Infectious Paralysis of Guinea-pigs

This disease occurs sporadically in guinea-pigs, and closely resembles poliomyelitis. According to Römer (1911), who first described it, it can be transmitted to normal guinea-pigs by the intracerebral inoculation of filtered or unfiltered brain or cord suspensions. After an incubation period of 9 to 22 days, the animals become febrile and lose weight; nervous disturbances develop, consisting of a gradually increasing muscular weakness, particularly of the hinder parts of the body; paralysis of the hind legs develops, and occasionally of the bladder. The disease lasts from 3 days to 3 weeks. Pathologically, the lesions are those of a disseminated meningo-myeloencephalitis. They differ from those of poliomyelitis in showing greater meningeal infiltration, and in being most severe around the central canal of the cord. The virus is constantly present in the central nervous system; it is also found in the prevertebral, inguinal, and mesenteric lymph glands, and occasionally in the liver and spleen.

BENIGN LYMPHOCYTIC CHORIOMENINGITIS. PSEUDO-LYMPHOCYTIC CHORIOMENINGITIS. SWINEHERDS' DISEASE

These three diseases may be considered together. In 1925 Wallgren coined the term " acute aseptic meningitis " to comprise cases of an acute febrile disease, accompanied by meningeal irritation and sometimes by inflammation of the upper respiratory tract, terminating favourably within 10–14 days. Since then, three different viruses capable of giving rise to a benign aseptic meningitis have been described: (1) the virus of benign lymphocytic choriomeningitis, which has been found mainly in the United States; (2) the virus of pseudo-lymphocytic choriomeningitis, which has been isolated on only two occasions—both in this country; and (3) the virus of swineherds' disease, which, so far, appears to be confined to France, Switzerland and Italy. These three viruses account for about one-third

of the cases of acute aseptic meningitis; the ætiology of the remainder is unknown (Report 1942).

Benign Lymphocytic Choriomeningitis.

Armstrong and Lillie (1934), while investigating a fatal case of St. Louis encephalitis, isolated a virus, quite different from the ordinary St. Louis virus, which gave rise in experimentally inoculated monkeys and mice to a lymphocytic choriomeningitis. An apparently identical virus was isolated by Scott and Rivers (1936) (see also Rivers and Scott 1936) from two benign cases of non-bacterial lymphocytic meningitis in adult males in the United States, and by Findlay, Alcock, and Stern (1936) in this country from the cerebrospinal fluid of two patients who exhibited vague nervous symptoms following a febrile attack. A similar or identical virus was isolated by Findlay and his colleagues from an irradiated mouse showing nervous phenomena. Traub (1936*a*, *b*, 1938, 1939) established the existence of an endemic infection with the same virus in a colony of normal mice, and showed that infection occurred either *in utero* or shortly after birth.

In man, the disease is uncommon during the first decade. Males and females are equally affected. Most cases occur in the winter and spring (see Blackfan 1941). The incidence is not known, but judged by the finding of neutralizing antibodies in the blood serum of patients who have recovered from catarrhal infections of the upper respiratory tract without meningeal symptoms, and of apparently normal persons, the infection would appear, in the United States at least, to be fairly widespread (see Armstrong and Wooley 1937, Wooley *et al.* 1939). A history of an influenza-like attack, followed in ten days or so by meningeal symptoms, is suggestive of the disease. Though the virus has been demonstrated once in the human nasopharynx by MacCallum and Findlay (1939), it is by no means certain that infection is spread by droplets. On the whole, the evidence suggests that man becomes infected from grey mice (*Mus musculus*), which are known to excrete the virus in the urine, faeces, semen, and nasal secretions (see Traum 1936*b*, Armstrong and Sweet 1939, Armstrong *et al.* 1940). A few laboratory infections have been reported.

The disease can be transmitted to mice, guinea-pigs, monkeys, and human beings.

Five to twelve days after intracerebral inoculation *mice* become ill, develop tremors of the limbs, and die in 1–2 days with spastic convulsions of the hind legs. Inoculated subcutaneously or intraperitoneally they develop an inapparent infection followed by immunity (Ronse 1937). *Guinea-pigs* develop a febrile reaction after intracerebral, intranasal, or subcutaneous inoculation, lose weight, and often die, though not before the 12th day. Meningitis is uncommon, but pneumonia may occur. The spleen and suprarenal glands are said to be most heavily infected, the liver and lungs less so (Mendoza 1937). According to Shwartzman (1944) the virus, in mice and guinea-pigs, is said to be associated with the red blood corpuscles. In *monkeys*, the virus has been shown to be widely disseminated throughout the tissues; pathologically, areas of lymphocytic infiltration are found in most of the organs (Armstrong *et al.* 1936, Lillie 1936). Not all strains are pathogenic for guinea-pigs. The same applies to rats. Rabbits and pigs are insusceptible. Lépine, Mollaret and Kreis (1937) have described the effect of inoculating *human beings*. The subcutaneous injection of 2 ml. of virulent mouse brain was followed in 36–72 hours by a benign and almost symptomless fever of an undulating type lasting up to 3 weeks. Two or three waves occurred, separated by apyrexial intervals. In about half the patients the last wave of the fever was accompanied by a meningeal reaction, starting abruptly and characterized by headache, sometimes vomiting, and by

contractures with a positive Kernig sign. The symptoms began to disappear in 2–3 days.

The virus seems to be about 37–60 mμ in diameter (Scott and Elford 1939). It has been cultivated successfully on the chorio-allantoic membrane of the developing chick embryo (Bengtson and Wooley 1936), and in a simple minced chick embryo serum medium (MacCallum and Findlay 1940). In 50 per cent. neutral glycerol phosphate solution at 0° C. it may remain alive for a few months, and also when dried *in vacuo* and kept at 0° C. in sealed ampoules, but it dies rapidly under other conditions. A suspension kept at 20° C. was found to be non-infective within 3 hours (Lépine, Sautter and Kreis 1937). A relatively heat-stable soluble antigen of protein nature can be demonstrated in the virus by precipitin and complement-fixation reactions (Smadel *et al.* 1939, 1940). It appears to be different from the antigen that reacts with neutralizing antibodies in immune serum.

Diagnosis.—The cerebrospinal fluid does not clot; the sugar and chlorides remain normal; and the fluid contains a considerable excess of lymphocytes. The virus may be demonstrated in the blood, or in the cerebrospinal fluid soon after the onset of meningeal symptoms, by inoculation intracerebrally into mice derived from a known non-infected stock, and its identity may be determined by neutralization with specific sera or by a specific complement-fixation reaction (Smadel and Wall 1941). Indirectly, the diagnosis may be assisted by the demonstration of complement-fixing antibodies (see Casals and Palacios 1941) or of neutralizing antibodies (Armstrong and Wooley 1935, Wooley *et al.* 1937) in the patient's serum. Antibodies appear to be somewhat variable in their appearance, and there is some evidence to suggest that complement-fixing antibodies appear rather earlier than neutralizing antibodies.

Pseudo-lymphocytic Choriomeningitis.—Only two cases of this disease have so far been described. Clinically, they resembled benign lymphocytic choriomeningitis. MacCallum and his colleagues (1939) isolated a virus from the cerebrospinal fluid of each of the patients. It was considerably larger in size—150 to 225 mμ—than the true choriomeningitis virus. It gave rise in mice, guinea-pigs, and *rhesus* monkeys to a clinical and pathological picture very similar to that of choriomeningitis, except that in mice inoculated intracerebrally the incubation period was shorter—4 to 5 days instead of 5 to 12 days—and the pathological lesions were less extensive. Judged by cross-immunity tests in mice, the two viruses appeared to be distinct.

Swineherds' Disease.—A disease affecting swineherds and occurring in Haute-Savoie, Switzerland, and Italy, was described in 1914 by Henri Bouchet under the name of pseudo-typhoid-meningitis. From the observations of Durand and his colleagues (1936, 1937, 1938) and of Campanacci (1939) there seems little doubt that the disease is due to a filtrable virus, and that infection occurs from pigs to man—possibly by the rubbing of infected fæces into the skin. After an incubation period given variously as 2–4 weeks and 5–8 weeks, the disease begins with fever, headache, and severe muscular pains. Digestive troubles appear—foul breath, green vomit, abundant fœtid diarrhœa, meteorism, and sometimes rectal hæmorrhage. After about four days of this preliminary typhoid state, the temperature falls to normal, but in two days or so it rises again and meningeal symptoms appear. The cerebrospinal fluid is under considerable pressure and the lymphocytes usually number several hundred per c.mm. After a further three or four

days the meningeal phase comes to an end and the fever falls by crisis or lysis. Conjunctivitis and a maculo-papular rash may be seen in some cases. The disease is practically never fatal, and leaves behind it a fairly solid immunity. The blood is infective during the first week, and the urine during the second week and early stage of convalescence. The infectivity of the cerebrospinal fluid is variable. The virus, which passes through an L2 candle, can be transmitted to swine, cats, ferrets, rats, mice, and guinea-pigs. Experimental inoculation of man is followed, after an incubation period of about 12 days, by a disease similar to, but on the whole milder than, the natural disease. In *pigs*, the natural disease is characterized by fever, digestive disturbances, salivation, stiffness of the neck, muscular spasms, circular movements, and often terror of food. The case mortality is very low. At post mortem punctiform hæmorrhages are found in the intestine, renal pelvis, brain and throat. The disease may occur in epidemic form. Young pigs, 1–2 months of age, are chiefly affected.

RABIES

Rabies is a disease of animals affecting particularly dogs, cats and carnivorous wild animals; herbivora are less frequently attacked. The disease is transmissible to human beings by the bites of rabid animals, notably dogs, cats, wolves, and jackals. The incubation period varies according to the age of the animal, the situation of the bite, and the severity and nature of the bite. Generally speaking, it varies in animals from 2 to 8 weeks, and in human beings from 6 to 8 weeks; it tends to be shorter in young animals and children, and after severe lacerated or deep punctured wounds about the head, face or neck. The disease does not uniformly appear after infection; according to Hutyra and Marek (1926), only 30–40 per cent. of dogs bitten by rabid animals develop the disease. In human beings the proportion of persons bitten by rabid dogs who die of rabies or contract the disease is given by Cornwall (1923) as 35 per cent. and by Hull (1941) as 16 per cent. The reasons for this are not clear; it seems probable, however, that the body is able to withstand small doses of the virus, especially if the virus does not come into intimate contact with exposed nerves. Once the disease has developed, it is almost always fatal.

Sometimes the rabies virus gives rise in human beings to an acute *ascending myelitis*. The first case of this type was recorded by Knutti (1929), who based his diagnosis on the presence of Negri bodies in the ganglion cells of the spinal cord, and on the development of rabies in inoculated rabbits. A very remarkable outbreak of the disease was observed by Hurst and Pawan (1931) in Trinidad. Besides the occurrence of ascending myelitis in human beings, a form of paralytic rabies, erroneously diagnosed as botulism, was also met with in cattle. Investigation failed to disclose any history of a bite by a rabid animal, but evidence was brought to show that both the human and the cattle disease was due to vampire bats which conveyed the disease by sucking the blood of their victims (see also Pawan 1936). Further observations show that paralytic rabies is apparently widespread among animals in Brazil, where infection is carried by the vampire bat *Desmodus rufus* (de Verteuil and Urich 1936). On cattle ranches the disease may reach epidemic proportions (Molina 1938). In South Africa the dog appears to play a negligible rôle in the perpetuation of infection. The main carriers are wild carnivora of

the mongoose family, particularly the meercat *Cynictis penicillata* (see Snyman 1940).

Animal Inoculation.—Rabies is transmissible to animals by subdural, intracerebral, or intravenous inoculation ; subcutaneous and cutaneous inoculations are less regular in their results. Pasteur and his colleagues (1881) found that dogs, when inoculated into the subarachnoid space over the brain with a suspension of the brain of a rabid dog, developed rabies after an incubation period of 1 to 2 weeks, and died within 3 weeks. If the virus is inoculated subdurally into a rabbit, the incubation period of the disease is about a fortnight ; but if the virus is passed in succession through a series of rabbits, the incubation period gradually falls, till after 20 to 25 passages it is only 8 days, and after a further 20 to 25 passages it is only 7 days (Pasteur 1885). The effect of passage is to convert the original " street virus " into a " fixed virus." These two viruses differ in certain respects. Thus, according to Levaditi, Nicolau and Schoen (1924), the fixed virus has a greater affinity for the nervous system of the rabbit than the street virus ; allied with this increased neurotropism, is the failure of the fixed virus to give rise to Negri corpuscles. Inoculated on to the scarified skin of the rabbit, both viruses give rise to rabies ; but if they are injected subcutaneously, the street virus alone proves virulent. It would appear that by passage through the rabbit's brain, the fixed virus has acquired a strong neurotropic affinity, which enables it to give rise to rabies in 7 days after subdural injection, but which usually renders it avirulent on subcutaneous inoculation. The street virus, on the other hand, takes about 14 days to produce rabies on subdural inoculation, and usually proves virulent when introduced subcutaneously. Fixed viruses can likewise be produced by passage through guinea-pigs, dogs, hens, and monkeys (Pasteur *et al.* 1884). Both street viruses, and the fixed viruses derived from them, show considerable variability in their virulence for animals ; some rarely produce infection when inoculated subcutaneously, others always do so (Marie *et al.* 1927). Pasteur (Pasteur *et al.* 1884) found that by passage through the brain of monkeys, the virus diminished in virulence for dogs, though when passed through rabbits or guinea-pigs it uniformly increased in virulence.

The virus is present in the saliva of rabid animals, and has sometimes been demonstrated in it 4 days before the onset of clinical symptoms. It is also found in the central nervous system, the peripheral nerves, and in the salivary glands, less regularly in the cerebrospinal fluid. Occasionally it may be demonstrated in the blood and the internal organs (Pasteur *et al.* 1884, Remlinger and Bailly 1931, 1932). The virus passes along the nerves from the local injury, and reaches the central nervous system ; this is shown by the fact that (1) the animal may live after resection of experimentally infected nerves ; (2) the resected nerves are themselves infective ; (3) the portion of the spinal cord in connection with the infected nerve becomes infective before other parts of the central nervous system (di Vestea and Zagari 1889, Marie *et al.* 1927). According to Nicolau and Galloway (1928), the virus, when introduced into the brain, may spread centrifugally, and be found in such nerves as the brachial and sciatic ; the passage of the virus along the nerves sets up an interstitial neuritis. It has been suggested that the salivary glands owe their infectivity to the presence in them of neurones, which may occur as single cells or as ganglionic aggregations, and which are situated just under the epithelium ; if the epithelium is abraded, the virus is set free from the neurones, and infects the saliva (Manouélian and Viala 1927, 1928, for references see McKendrick 1928*b*).

Properties of the Virus.—The rabies virus can pass through Berkefeld candles. Galloway and Elford (1936), from filtration experiments carried out with gradocol membranes on a strain of fixed virus, estimate its size as 100–150 mμ. This agrees with the value obtained for another strain of fixed virus by Yaoi, Kanazawa and Sato (1936). The virus can be grown in tissue cultures containing embryo brain and human or monkey serum (Kanazawa 1936, Webster and Clow 1937). It can also be cultivated on the chorio-allantoic membrane of the developing chick embryo, provided 5 or 6-day embryos are used (Kligler and Bernkopf 1941). Cultivated strains tend to lose their virulence fairly rapidly. According to Sankaran, Iyengar and Beer (1934), the virus can be separated from infected rabbit

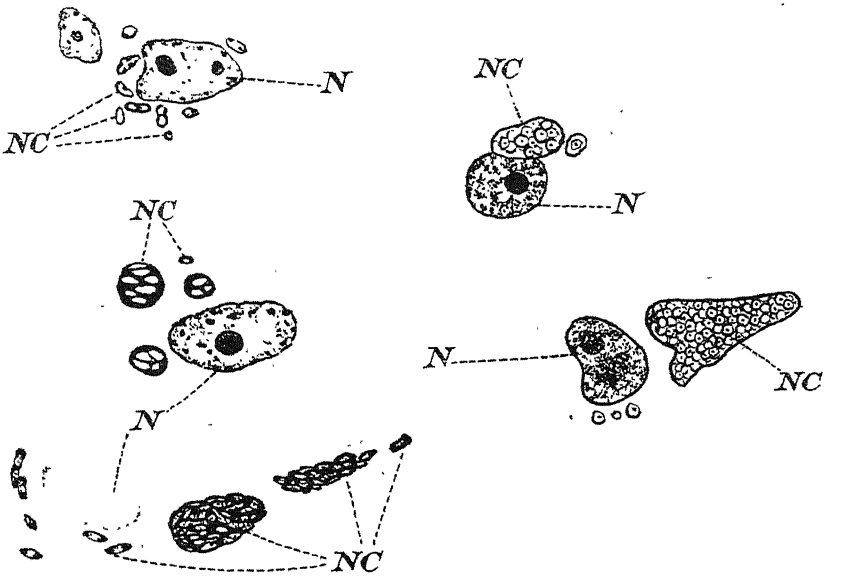


FIG. 302.

Negri corpuscles in Ammon's horn. N = nucleus. N.C. = Negri's corpuscle. (After Manouélian, and Manouélian and Viala.)

brain by electrophoresis. It is not very resistant. It is destroyed by exposure to a temperature of 60° C. for 30 minutes. Slow drying gradually renders it inert, but if rapidly dried it may retain its infectivity for months. The fixed virus is very sensitive to the photodynamic action of methylene blue (Shortt and Brooks 1934, Galloway 1934) and to irradiation with ultra-violet light (Sankaran and Beer 1935). It is readily killed by bile. Little is known about its antigenic structure, but most strains seem to resemble each other fairly closely (Remlinger and Bailly 1930, Havens and Mayfield 1933).

One of the characteristic properties of the street virus is its power of giving rise to inclusion bodies in the nerve cells, known as Negri corpuscles (Negri 1903*a*, *b*) (Fig. 302). They are found most frequently in the cytoplasm and dendritic prolongations of the nerve cells, particularly in those of Ammon's horn. Usually they

are about 3–10 μ in diameter, but they vary from 1 to 27 μ (Manouélian 1912). They generally conform in shape to their situation in the nerve cell, being rounded when in the cytoplasm, oval when compressed by the nucleus, and fusiform when in the dendrons. Each Negri body consists of a homogeneous mass containing clearer corpuscles, which may themselves contain one or more corpuscles, and sometimes short rods or granules. When stained by Giemsa, the homogeneous ground substance is lightish blue; the larger corpuscles are pink, and the small corpuscles, rods, or granules are red or reddish-violet in colour. Fine blue-staining granules, which Negri regarded as forming a diffuse nucleus, are scattered throughout the body. In infections with fixed virus, Negri bodies are not usually present, but instead very fine granules, staining red with Mann's method, may be found in enormous numbers in the nerve cells (Manouélian 1912). The Negri bodies vary considerably in appearance. There is little doubt that they represent intracellular micro-colonies of the virus. Their presence in nervous tissue is diagnostic of infection, and their demonstration either directly, or indirectly by the mouse passage method (Webster and Dawson 1935), affords the most rapid means of determining the presence of infection in a suspected animal.

Diagnosis.—In attempting to reach a diagnosis of rabies in a human being before the disease has declared itself, it is important if possible, not to kill the animal responsible for the bite, but to keep it in safe captivity for a few days to see whether the typical symptoms of the disease develop. If it is killed before the onset of the paralytic stage, no Negri bodies may be found in the brain. Once the animal is paralysed, it should be killed, and the brain examined by a rapid section method for Negri corpuscles. If the animal has been prematurely destroyed, or if the brain has been improperly preserved, resort should be had to the mouse inoculation test described by Webster and Dawson (1935).

Webster (1936) recommends that sterile pieces of Ammon's horn of the suspected animal should be made into a 5 or 10 per cent. suspension with distilled water, and that 0·03 ml. should be inoculated intracerebrally and 0·25 ml. intramuscularly into each of 6 mice belonging to the specially susceptible Swiss breed. One animal should be sacrificed on the 5th, 6th, and 7th days respectively, and its brain examined for Negri bodies. The remaining three are observed for 4 weeks for signs of rabies. In practice, a negative diagnosis may usually be given in 3 weeks (Thomas 1939). The advantage of the mouse over the rabbit is that it is more susceptible, it is easier to inoculate intracerebrally, and it develops rabies, on the average, a week earlier.

If the brain of a suspected animal cannot be examined fresh, it should be packed in ice before transmission to the laboratory; or, alternatively, half of it may be sent in 10 per cent. formalin for section and half in 50 per cent. glycerol for inoculation. It is possible that the complement-fixation test may be found of value in diagnosis (Casals and Palacios 1941), but so far little work has been carried out on it.

Prophylactic Treatment of Rabies.

After infliction of the bite, an attempt should be made to destroy as much of the virus in the wound as possible. For this purpose, treatment with a 20 per cent. solution of soft soap is said to be as effective as cauterization with fuming nitric acid (Shaugnessy and Zichis 1943). Tincture of iodine is also useful, if applied within 30 minutes.

Rabies is a fatal disease, and once symptoms have declared themselves, treat-

ment is of no avail. But the disease is characterized by a long incubation period ; and it was Pasteur (1885) who realized the possibility of utilizing this period for carrying out specific vaccine treatment. Pasteur found that the spinal cords of rabbits dying of experimental rabies gradually lost their virulence when dried in air over KOH. By inoculating dogs with cords dried for varying periods, beginning with the least virulent and working up to the most virulent, he was able to immunize them to both natural and experimental rabies. His first human patient was a boy, Joseph Meister, aged 9 years, who had been bitten by a rabid dog in 14 places. Treatment was commenced 60 hours after the bite. Subcutaneous inoculation was performed, starting with a cord that had been dried for 14 days, and working up to a cord only 1 day old ; altogether 13 injections were given within 11 days. Rabbits inoculated with the cords 7 to 14 days old remained well ; rabbits inoculated with the cords 1 to 6 days old died after an incubation period increasing from 7 to 14 days. The cords first injected were therefore avirulent ; those used for the later injections were virulent. The treatment was a complete success, and formed the starting point of a system of preventive inoculation that is of world-wide distribution.

Pasteur's original method of inoculation is still used to-day, but a number of other methods have been developed by different workers. The most important of these are : (1) Högyes' method ; this consists of the subcutaneous injection of 1/100 to 1/10,000 dilutions of a 1 per cent. suspension of fixed virus of full virulence. This method has been used extensively with good results ; occasionally, however, the fixed virus may cause infection. (2) Babes' method ; in this the spinal cord is heated to 80°, 60°or 45° C. for 10 minutes, in order to attenuate the virus to varying degrees. (3) Semple's method ; the virus is killed with 1 per cent. carbolic acid. (4) Alivisatos' (1922, 1926) method ; the virus is attenuated with ether. (5) Fermi's method of sero-vaccination. Fermi (1926) prepares his vaccine by exposing a 5 per cent. suspension of fixed living virus to 1 per cent. phenol for 1 to 10 days. The serum is prepared by the immunization of horses with the vaccine. The vaccine and the serum are mixed in varying proportions, and injected twice daily.

It is very difficult to ascertain which of these methods is of most value. McKendrick (1940), who has reviewed the results furnished over a number of years by the Pasteur Institutes in different countries, comprising a total of over one million persons treated, finds that there is very little to choose between them. From a careful analysis of the figures he regards it as improbable that any of the vaccines differs in efficacy from the rest by more than 25 per cent. On the other hand, paralyses resulting from the vaccine itself were observed about three times as often in patients treated by Pasteur's original method or by Högyes' dilution method as in those receiving vaccines killed by phenol, ether, or heat. Paralyses of this type constitute one of the objections to the treatment. They occur about once in every 3,000 to 10,000 patients, according to the type of vaccine used. The commonest time for them to appear is between the 11th and 30th days after the bite, that is to say, considerably before the onset of rabies in untreated persons. They prove fatal in about 25 per cent. of cases. What they are due to is not yet known ; for a discussion of the subject see Remlinger (1927).

The average case mortality in treated patients in McKendrick's series varied over a series of years between 0·23 and 0·49 per cent.

How, it may be asked, can a dead vaccine, inoculated into the patient after living virulent virus has gained access to the body, afford any protection against

the disease? The answer to this question is still being sought. The virus is known to be neurotropic, and almost certainly finds its way to the brain and cord by way of the nerves. If an attenuated virus was inoculated in enormous quantities very soon after the bite, it is conceivable that some of it might reach the susceptible nerve cells and block the entry of the virulent virus. But how can this happen with a dead vaccine, unless transport along the neurones is purely mechanical? Yet, from McKendrick's figures a dead vaccine appears to be just as efficacious as a living attenuated virus. Moreover, there seems to be no advantage in early treatment; the mortality of those treated 0–4, 5–7, or 8–14 days after the bite was, among Europeans, 0·19, 0·10, and 0·06 per cent. respectively. Animal experiments do not increase our confidence in vaccination. The observations of Shortt and of Covell and their colleagues in India (Shortt *et al.* 1934, 1935, Covell *et al.* 1936), and of other workers (see Webster 1939*b*) seem to show that (1) no vaccine can protect an animal *after* exposure to infection by rabies virus, (2) vaccine given *before* exposure to infection is effective only provided (*a*) it is given repeatedly and in large doses, totalling at least 1 per cent. of the body weight, and (*b*) the test dose is given subcutaneously, intramuscularly, or by some other route than the intracerebral.

Considerations such as these have led many workers to question the value of the vaccine treatment of rabies. Ever since Pasteur recorded his apparently brilliant successes, persons who have been bitten by rabid animals have been given treatment as a matter of course. In such a disease it would be unjustifiable to withhold it so long as there was any reason to believe that it might confer some degree of protection. The result has been that there are no adequate control figures available. A high proportion of persons treated have probably not been bitten by rabid animals, and, of those who have, probably not more than a quarter would have developed rabies anyway. The figures of the Pasteur Institutes therefore throw little light on the main question at issue.

Progress in the past has been hampered by the failure of a satisfactory technique for standardizing anti-rabic vaccines. The mouse inoculation test, however, introduced by Webster (1939*a*) promises to be of value for this purpose. By its use Webster found that commercial vaccines of the killed type were often devoid of protective action or were effective only in doses greater than those recommended by the manufacturers (see also Wyckoff and Beck 1940, Habel 1940*a*). Habel (1940*b*), moreover, has noted considerable variations in the antigenic potency of the different strains used to prepare commercial vaccines. In an effort to produce a more reliable product, Webster and Casals (1942*a*, *b*) have prepared a vaccine from infected dog brain rendered non-virulent by exposure to ultra-violet light. In the laboratory favourable results were obtained in the protection of mice and dogs, but its use on a field scale under properly controlled conditions will have to be awaited before an opinion on its value can be expressed.

The Paris Conference on rabies recommended that prophylactic treatment should be applied to all human patients whenever they had been bitten, or whenever a mucosa had been contaminated with rabid saliva, even though no wound had been inflicted. The treatment should be commenced as soon as possible after the bite. In countries where rabies is common, they recommend that all dogs should be vaccinated with a single dose of killed or of fixed virus, and revaccinated every year. But a dog or other animal that has been bitten by a certainly rabid animal must be killed, whether vaccinated or not; if bitten by a suspect animal, then

the dog may be vaccinated therapeutically, and kept in quarantine for 6 months. The measures they recommend for the elimination of rabies consist in restricting all dogs not fitted with muzzles to the homes of their owners, and in destroying all stray dogs. In this country, where the muzzling orders and the strict supervision of all imported dogs have been carried out when need has arisen, rabies is non-existent.

How far prophylactic inoculation of dogs is effective in practice, it is impossible to say. Gautier (1940–41), who reviewed the evidence, was able to reach no definite conclusion. Webster (1939*b*) found that a single dose of the usual canine vaccines was ineffective, thus confirming the previous observations of Barnes and his colleagues (1930, 1934). Whether the ultra-violet irradiated vaccine of Webster and Casals (1942*a*, *b*) will prove more efficacious, it is as yet too early to say. For much useful information on rabies, the reader is referred to the monograph by Remlinger and Bailly (1938*a*).

Pseudorabies

Synonyms: Mad itch: Infectious bulbar paralysis: Aujeszky's disease.

This disease, which was described by Aujeszky in 1902, has only recently attracted much attention. It appears to be an acute infection of the central nervous system affecting dogs, cats, cattle, horses, pigs, goats, sheep, rats, and mice, but not man. Though originally met with in Hungary, it has now been reported from a number of other European countries and from both North and South America. Unlike rabies the incubation period is very short—only a matter of hours—the onset is brusque, there is intense pruritus, which often completely dominates the clinical picture, the mental faculties are preserved, paralysis occurs very late and does not involve the lower jaw, the disease lasts only 24–48 hours, and sudden death is common (Remlinger and Bailly 1934). Except in pigs, the case-mortality rate is usually very high. The mode of infection is still in doubt. According to Shope (1935), the disease in cattle is very fatal but non-contagious, whereas in pigs it is relatively mild but highly contagious. Shope believes that in pigs infection occurs by the nose, and that the abraded skin of cattle can probably be infected from contact with the pig's snout. A study of neutralizing bodies in the serum suggests that pseudorabies is a highly prevalent but unrecognized disease in the hogs of the Middle West. There is some evidence that the disease is spread by rats, and that pigs may become infected by feeding on contaminated material.

The disease can be reproduced experimentally in most animals, though monkeys appear to be fairly resistant. In the rabbit, which is the most susceptible of the small laboratory animals, the disease exhibits a diverse and striking symptomatology. Infection is possible by practically any route. Intracerebral inoculation gives rise in 20–40 hours to a condition of wild excitement, accompanied by salivation, grinding of the teeth, blindness, and later coma and death. In rabbits injected subcutaneously the incubation period is 50–75 hours; the animals then start scratching and biting the site of inoculation; their efforts become increasingly savage till the skin is hairless, abraded, and bleeding; feebleness sets in, and death occurs in a state of collapse 6–24 hours after the onset of pruritus (Shope 1931). The clinical picture, however, is very variable, and may assume an encephalitic, pseudo-herpetic, meningeal, paralytic, pruriginous, fulminating, or even abortive form (Remlinger and Bailly 1934). Some animals can be infected by feeding.

The causative virus is widely distributed in the body, being often demonstrable in the blood and internal organs as well as in the central nervous system. It may be present in the saliva, but there is no evidence to suggest that the disease is transmitted by biting (Remlinger and Bailly 1937). In some animals it is said to be excreted by the urine. It can pass through a Berkefeld W filter, and has been found by the gradocol membrane technique to have a particle size of 100–150 mμ (Elford and Galloway 1936). It is killed by exposure to a temperature of 55°–60° C. in 35 minutes, but survives in 50 per cent. glycerol in the ice-chest for many months (see McKinley 1929). It is readily destroyed by bile. It has been successfully grown in tissue cultures containing rabbit or guinea-pig testicle and in a minced chick embryo medium (Traub 1933). It can also be cultivated on the chorio-allantoic membrane of the chick embryo (Mesrobeanu 1938); focal lesions are produced, and the embryo dies in 3–4 days (Glover 1939); the virus invades the central nervous system of the embryo (Bang 1912). Cross-immunity tests show that it is distinct from the virus of rabies (Gerlach and Schweinburg 1935), and from the herpes virus (Nicolau *et al.* 1937), which in many ways it resembles (see King 1940). Immune sera that neutralize the virus can be prepared by inoculation of guinea-pigs (Shope 1931). Antigenically, only one type of virus has so far been recognized (Remlinger and Bailly 1937).

Histologically, the picture varies in different animals. In general, gross lesions are few. Hyperæmia of the meninges and small hæmorrhagic extravasations may be seen. In rabbits and guinea-pigs the virus gives rise to local inflammation and necrosis at the site of inoculation, passes up the peripheral nerves as evidenced by the presence of intranuclear inclusion bodies and causes degeneration of the nerve and glial cells in the spinal ganglia and segments of the cord corresponding to the peripheral nerve invaded (Hurst 1933). The lungs may show intense hæmorrhagic lesions, containing virus in considerable quantity (see Galloway 1938). In cows, primary degeneration of the nerve and glial cells occurs in the cerebral cortex, especially Ammon's horn.

Animals that recover from an attack of the disease possess a strong immunity even against intracerebral inoculation (Gerlach and Schweinburg 1936); but all attempts so far to immunize healthy animals by vaccination have proved a failure. For a general description of the disease and its causative virus the reader is referred to an article by Galloway (1938) and to the monograph by Remlinger and Bailly (1938*b*).

Scrapie

Synonym: French. *Tremblante du mouton*

Scrapie is a disease of sheep that occurs in Great Britain - mainly the northern part—and on the continent of Europe. The onset is often insidious and is characterized by undue excitability and jerky movements of the ears, eyelids, lips and limbs. Later, irritation of the skin occurs, starting at the root of the tail and gradually becoming generalized. Itching is intense and almost intolerable. Emaciation rapidly sets in, the animal becomes weaker and weaker, and dies in a fortnight or so. From start to finish the disease lasts 3 to 4 months. In the so-called "trotting" variety, the itching is less severe, but the animal periodically drops every now and again, as if dead, and then rises in a few minutes and resumes its trot. Pathologically, no characteristic lesions have been found. Early attempts

to transmit the disease by inoculation failed, possibly because the animals were not observed long enough. More recently, Cuillé and Chelle (1936, 1938) have shown that the brain and spinal cord are infective, and that the disease may be reproduced in susceptible sheep by subcutaneous, intra-ocular, epidural, or intracerebral inoculation. The incubation period, however, is very long; it is seldom less than a year and may extend nearly to 2 years. The infective agent appears to be filtrable, as a suspension of cord is still virulent after passage through a Chamberland L3 candle.

We append brief descriptions of two animal diseases whose position in our rough scheme of classification is very doubtful.

Salivary Gland Virus of Guinea-pigs

Cole and Kuttner (1926) observed greatly swollen epithelial cells in the salivary glands of guinea-pigs; they were found in 84 per cent. of full-grown animals. Injection of a suspension of the affected submaxillary glands intracerebrally into young guinea-pigs was followed by fever, cerebral irritation, and death in 5 to 7 days, with diffuse subacute meningitis. In the exudate large numbers of cells were found similar to those in the salivary glands. Similar cells were also seen in the lesions resulting from inoculation of the same suspension into the testicle, lung, tongue, and submaxillary glands of young guinea-pigs. Markham (1938) has recorded the occasional finding of characteristic inclusions in the epithelium of the convoluted tubules in the kidney of naturally infected animals. The virus is destroyed at 54° C. in 1 hour; it survives for 11 but not for 28 days in 50 per cent. glycerol. It passes through a Berkefeld N candle. The virus appears to give rise to no clinical symptoms in naturally infected guinea-pigs. Infected guinea-pigs are immune to the intracerebral inoculation of virus; in the serum of these animals neutralizing bodies can be demonstrated (Andrewes 1930). More recently Kuttner and Wang (1934) have described the presence of acidophilic intranuclear inclusion bodies in the submaxillary glands of hamsters, white mice, and wild rats; each of these animals appears to be infected by a specific virus. Similar inclusion bodies have also been noted in the salivary glands of human infants, but animal transmission experiments have proved unsuccessful.

Pappenheimer and Slanetz (1942) have described a generalized visceral disease of guinea-pigs characterized by intranuclear inclusions of the herpes type. Macroscopically, areas of greyish-white necrosis were found in the liver but, microscopically, lesions were present in the lungs, spleen, and kidney as well. Infection was not transmissible experimentally, and the relation of the disease to that caused by the salivary gland virus was not clear.

Periodic Ophthalmia of Horses

This disease is characterized by a recurrent serous uveitis, with an acute onset, subsidence in 4–10 days, and a remission after 3–6 weeks. The symptoms can be reproduced in horses by inoculation of a Berkefeld N filtrate of infective material into the vitreous humour. Rabbits are also susceptible (Woods and Chesney 1930).

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CHAPTER 88

FILTRABLE VIRUS DISEASES—*Continued*

D. GROUP CHARACTÉRIZED BY CATARRHAL OR GENERALIZED INFECTION

MEASLES

MEASLES is a highly infectious disease of human beings, which is endemic throughout the world. In Europe and America epidemics tend to occur in alternate years, as a fresh susceptible population of children grows up. Among civilized peoples it is chiefly a disease of childhood, relatively mild in itself, but when complicated by respiratory infections—as it so often is—attended by a high mortality. Introduced, however, into non-endemic areas it affects young and old alike, and takes on a more malignant character. When it was imported, for example, into the Fiji islands in 1875, it carried off 20–25 per cent. of the entire population (see Brincker 1938). Most children exposed to infection for the first time contract the disease, but there is evidence that during epidemics a small proportion develop a latent infection resulting in a temporary or sometimes permanent immunity (see Halliday 1928, Stocks and Karn 1928, Enders 1941). In this country measles is a disease of winter and spring, the maximum mortality usually being recorded in April.

Anderson and Goldberger (1911) first recorded evidence indicating that measles could be experimentally transmitted to *monkeys*. The experiments of Nicolle and Conseil (1911, 1920) likewise suggested that monkeys were susceptible. The most convincing evidence, however, was brought by Blake and Trask (1921). These workers were able, by the intratracheal inoculation of filtered and unfiltered nasopharyngeal washings taken from measles patients 6 days before to 22 hours after the appearance of the rash, to produce in monkeys (*Macacus rhesus*) a disease closely simulating human measles. After an incubation period of 6 to 10 days, there developed a characteristic group of symptoms characterized by listlessness, drowsiness, leucopenia, catarrhal conjunctivitis, a rash on the labial mucosa, and a skin rash consisting of discrete red maculo-papules; fever was inconstant. Recovery occurred after an illness of 7 to 10 days; no respiratory complications were observed. The disease was transmitted from monkey to monkey by intratracheal inoculation of tissue suspensions (chiefly skin and buccal mucosa) of monkeys killed from 2–6 days after the onset of the reaction, and by intravenous inoculation with blood taken 7–13 days after infection; but it could not be carried on through monkeys indefinitely (Blake *et al.* 1936). Shaffer, Rake, Stokes and O'Neil (1941) inoculated children and monkeys—*Macacus mulatta*—simultaneously with blood drawn from human cases of measles in the early stages of the disease. Both sets of inoculations proved successful, the incubation period in the children varying from 5 to 12 days, in the monkeys from 6 to 16 days. The disease in the monkeys was generally milder than in the children,

and was not as a rule accompanied by either fever, cough, or coryza. Since Rake and Shaffer (1940) have found that nasopharyngeal washings are still infective after filtration through a Seitz EK disc, there seems little doubt that the disease must be due to a filtrable virus. As Enders (1941) points out, the monkey experiments would be more convincing if the disease could be transmitted back to man; so far this appears to have been done on only one or two occasions.

Numerous workers (Nevin and Bittman 1921, Duval and D'Aunoy 1922, Scott and Simon 1924, 1925) have claimed to have transmitted measles to *rabbits*. Their work is criticized by Purdy (1925), who completely failed to obtain any significant reaction in rabbits—even with material that was shown to be infective for monkeys. Taniguchi and his colleagues (1935) recorded the successful transmission of infection to *rabbits* and *guinea-pigs*, but there is reason to believe that the virus they established was not the measles virus, but one of the animal pox group (see Enders 1941). The evidence, on the whole, does not suggest that the disease has ever been successfully reproduced in animals, apart from monkeys.

Little is known about the virus.

Several workers have reported its cultivation on the chorio-allantoic membrane of the developing chick embryo, but most of the reports are unconvincing. More recently Rake and Shaffer (1940) state that they have been able to carry the virus through 20 serial egg passages and to reproduce infection with it in monkeys. Stokes and his colleagues (1943) were apparently able to transmit the disease in a modified form to children with virus that had reached its 66th serial egg passage, suggesting that the virus had undergone considerable attenuation in culture. Its growth in a tissue culture medium, consisting of Tyrode's solution, monkey serum, chick embryo tissue, and chicken plasma, has been reported by Plotz (1938) and confirmed by Rake, Shaffer and Jones (1941). In nasopharyngeal washings the virus is said to survive for several days at 0° C. or below, and to withstand 10 per cent. ether for at least 40 minutes (Rake and Shaffer 1940). Intranuclear inclusion bodies have been described by Broadhurst and her colleagues (1937, 1938) in the nasal mucosa, Koplik's spots, and white blood cells of measles patients, but their findings still await confirmation.

Prophylaxis.—General measures include notification, exclusion of children from school for a fortnight, and sometimes quarantine of non-immune contacts for 16 days. School closure is rarely called for; nor is it usually desirable to prevent healthy home contacts from attending school, provided that they are kept under observation. In view of the high complication rate due to cross-infection with hæmolytic streptococci, treatment at home rather than in hospital is advocated for patients with uncomplicated measles (see Wright 1945).

The prophylaxis of measles has been improved by the use of human *serum* from convalescent patients or from normal adults who have previously suffered from the disease. In children under 3 years of age, in weakly debilitated children, and in children suffering from some other serious disease, it is advisable to prevent an attack completely by injecting a fairly large dose of serum within 5 days of exposure to infection. The injection should be made intramuscularly, preferably into the vastus externus. The duration of the resulting immunity is 3–4 weeks, and the patient is subsequently susceptible to infection. After the age of 3 years it is desirable to aim, not at complete protection, but at attenuation of the attack. This is achieved by injecting the same quantity of serum within 6–9 days, or half the quantity within 1–5 days, of exposure to infection. The immunity following a modified attack appears to be permanent. The requisite dosage of serum is given in Table 191.

TABLE 191

DOSAGE OF SERUM IN MEASLES PROPHYLAXIS. (After Brincker 1936.)

Nature of Serum.	Limiting Dosage in ml.	Dosage Factor.	Route.	Prophylaxis.		Treatment.
				Protection.	Attenuation.	
Convalescent .	5–20	Age × 2	Intra-muscular	1–5 days	5–9 days	Within 6 days of onset.
Normal adult .	10–40	Age × 4	„	1–3 „	3–9 „	Dosage factor : age × 4.
Whole blood .	Double its dose as serum	Age × 4	„	1–3 „	3–9 „	Route intravenous.

Thus for a child under 3 years of age, 6 ml. of convalescent, or 12 ml. of normal adult, serum should be given for complete protection. For attenuation of the attack in a child of 6 years, 12 ml. of convalescent serum should be given between the 6th and 9th days after exposure, or 6 ml. between the 1st and 5th days. If adult serum is selected, double these quantities should be used. A fixed dose of 10 ml. of adult serum is used by some workers in preference to an age-adjusted dose ; if protection is desired it is given within the first 5 days, if attenuation is desired, within 6–9 days of exposure. Though this practice possesses the merit of simplicity it is doubtful whether it is altogether satisfactory (Gunn 1936). Convalescent serum should preferably be withdrawn from the donor 7–10 days after defervescence. Whole blood is of value mainly in general practice, when blood may be withdrawn from the mother or other member of the household and inoculated directly into the child.

For further information on the value of convalescent serum in prophylaxis reference may be made to the study of Stillerman, Marks and Thalhimer (1944).

Statistical analysis of the data so far accumulated has shown the undoubted value of serum prophylaxis (Report 1933, 1936, Hobson 1938). Convalescent serum is found to be more effective than normal adult serum, but only when administered to children under 5 years of age at an interval of less than 3 days after exposure to infection. It must be understood that to secure an attenuated attack, it is necessary not only to give serum, but also to make reasonably sure that the child has been adequately exposed to infection. Otherwise many children in whom it is desired to produce an attenuated attack resulting in lasting immunity may entirely fail to develop the disease and be left susceptible (Report 1936). For this purpose it is probably desirable to leave the child in contact with infection for a day or two and to delay giving serum for 5–9 days. (For further references to serum prophylaxis in measles see Zingher 1924, Debré *et al.* 1925, 1926, 1930, Copeman 1925, Toomey 1926, Haas and Blum 1926, Park and Freeman 1926, Benson and Lawrie 1927, Karelitz and Levin 1927, Kingsbury 1927, Gunn 1928, Benson 1929, Morales and Mandry 1930, Barenberg *et al.* 1930, Nabarro and Signy 1931, Gunn 1932, 1938.)

In place of serum the use of placental extract has been recommended by McKhann and Chu (1933) and McKhann (1937). In potency it occupies a place midway between convalescent and adult serum. The difficulty, however, of extraction of the placental globulins and the greater proportion of reactions to which it gives rise have not secured for it general acceptance.

The introduction of the gamma globulin fraction of human serum by Cohn and his colleagues (1944) bids fair to replace all other products in the serum prophylaxis of measles. The observations of Stokes, Maris and Gellis (1944) and of Ordman, Jennings and Janeway (1944) point to its high protective value; and the results obtained by Greenberg, Frant and Rutstein (1944) seem to show that a dose of 2 ml., given before the ninth day of exposure to infection, can be relied upon to afford complete protection to infants and children under 4 years of age. The reactions obtained to this "human immune serum globulin," as it is called, seem to be few and comparatively mild, and the small dosage required is likely to prove particularly attractive for the injection of infants and young children.

Unlike the Schultz-Charlton reaction in scarlet fever, no blanching of the morbilliform rash is produced by the inoculation of convalescent serum into the skin of a patient suffering from measles. If, however, 1–5 ml. of convalescent serum are injected subcutaneously at the beginning of the catarrhal stage, the appearance of the eruption is inhibited over an area some distance around the site of the needle puncture (Debré *et al.* 1923).

Vaccination against measles by an attenuated virus grown on the chorio-allantoic membrane of the developing chick embryo has been described by Maris and her colleagues (1943). A mild attack of the disease is said to be produced which apparently confers some protection against subsequent infection.

RUBELLA

SYNONYM: German Measles

Little is known about the causation of this disease. According to Hiro and Tasaka (1938) infection can be transmitted to susceptible children by subcutaneous inoculation of filtered nasal washings from patients in the acute stage. Habel (1942) has reported the reproduction of the disease in monkeys, *Macacus mulatta*, by the intranasal, subcutaneous, or intravenous inoculation of nasal washings or of blood taken within 24 hours of the appearance of the rash. After an incubation period of about 8–10 days the animals developed a leucopenia, fever, lymphocytosis, and a very light macular rash. Unfortunately, owing to circumstances, the nature of the experimental disease could not be proved by passage back to human beings. Some evidence was obtained to suggest that immunity following an attack may be slow in developing.

MUMPS

Mumps is an infectious disease of human beings characterized by a non-suppurative enlargement of the parotid glands, and sometimes of the testicles. It is commonest between 5 and 15 years of age, but often attacks young adult recruits to the fighting services. Most cases occur in the spring. The incubation period is usually 7–18 days. The disease seldom proves fatal, and even the encephalitis that occasionally accompanies it is usually benign. Second attacks are rare. Transmission appears to occur by droplets. There is some evidence that patients may be infective for a day or two before the parotitis becomes obvious. Isolation of patients should be maintained for a week after subsidence of the glandular swelling.

Though Kermorgant (1925) brought evidence to suggest that infection was caused by a spirochæte, there is now little doubt that the causative agent is a filtrable

virus. This conclusion, which was reached by Gordon in 1914 on the basis of monkey experiments, and by Wollstein in 1916 on the basis of experiments with cats, has received confirmation from the more recent experiments of Johnson and Goodpasture (1934, 1935), and Findlay and Clarke (1934). Saliva taken from patients in the early stage of the disease and inoculated into Stenson's duct, gives rise in *Macacus rhesus* monkeys to a disease similar to mumps. After an incubation period of 6–10 days or so, there is a rise of temperature, a leucopenia with a relative lymphocytosis, and a painful non-suppurative swelling of the parotid gland. Passage experiments can be made with a suspension of the ground-up glands filtered through a Berkefeld N candle. The intratesticular injection of such a filtrate gives rise in monkeys to a non-suppurative orchitis. Johnson and Goodpasture (1935) were able to transmit the disease back to human volunteers after it had been maintained for several generations in the monkey. Swan and Mawson (1943) succeeded in infecting monkeys by inoculation of cerebrospinal fluid from a patient suffering from encephalitis. According to Bloch (1937), the essential lesion of experimental parotitis in the monkey is focal acinar necrosis; œdema, hæmorrhage, and a lymphocytic reaction are said to be secondary manifestations. Transmission to rabbits has been claimed by many workers, but has so far not been confirmed (see Lépine *et al.* 1940).

The virus is resistant to freezing, drying, and glycerolation. It has not yet been cultivated, though mildly suggestive results on the chorio-allantoic membrane of the developing chick embryo have been reported by Swan and Mawson (1943).

According to Enders, Cohen and Kane (1945) complement-fixing antibodies appear in the serum of patients suffering from mumps and remain, though in diminishing quantity, for several months; their presence may be of value not only in the diagnosis of the parotitis itself, but in that of the meningo-encephalitis that sometimes occurs as a complication during or after an attack of mumps (Kane and Enders 1945).

Monkeys that have recovered from one attack of the disease are actively immune. Attempts to confer passive immunity to intraparotid infection by inoculation with the serum of persons who have recovered from mumps have so far proved a failure (Johnson and Goodpasture 1936). On the other hand, some degree of protection can be conferred on human beings by inoculation of convalescent serum within the first five days after exposure to infection. The dosage varies. Most workers suggest 2–3 ml., but Stillmunkès (1937) recommends 15–20 ml. The occasions on which serum prophylaxis of a non-fatal disease are required must be relatively few. For a general review of mumps, the reader is referred to the article by Wesselhoeft (1941).

YELLOW FEVER

Little was known about the ætiology of this once-dreaded disease till the studies of the American and the French Commissions were undertaken in the early years of this century.

The American workers, Reed, Carroll, Agramonte and Lazear (1900–01), at the beginning of the century, first showed that the blood of patients was infective during the first 3 days of the fever; that the infecting agent was able to pass through a Berkefeld filter; that the virus was destroyed in the blood by heating

to 55° C. for 10 minutes; that infection was not spread by contagion; that mosquitoes, *Aèdes ægypti* (also called *Aedes argenteus*, and *Stegomyia fasciata*) fed on patients during the first 3 days of the fever, became, after an interval of about 12 days, capable of transmitting the disease to normal persons; and that by the suppression of these insects, yellow fever could be brought under control.

Marchoux, Salimbeni and Simond (1903), likewise working with human volunteers, largely confirmed these results. They found, moreover, that the serum of patients during the first 3 days of the fever, but not later, proved infective to normal persons when injected subcutaneously in a dose of 0·1–1·0 ml.; that defibrinated blood kept at 24°–30° C. under a vaseline seal remained infective for 5, but not for 8 days; that the injection of blood kept for 8 days under these conditions was apparently able to produce some degree of immunity in human beings; and that the serum of convalescent patients appeared to be endowed with both prophylactic and therapeutic properties.

The natural outcome of these findings was to undertake an intensive anti-mosquito campaign. The result of this so far exceeded expectations that yellow fever was apparently wiped out of Central and South America. Ten years ago it was believed that the only serious remaining focus of infection was in West Africa. The work, however, of the past few years, carried out to a considerable extent under the auspices of the Rockefeller Foundation, has necessitated a complete revision of our ideas on the epidemiology of the disease. So far from having been wiped out in South America, it is now known that infection is widespread through large parts of the country, and that the disease occurs in an endemic form whose very existence had been previously unrecognized (Soper 1936, 1937, 1938). As has happened so often with other diseases, the more flagrant epidemic manifestations of the disease were allowed to obscure the less obvious but more persistent undercurrent of infection. The institution of routine post-mortem examinations of every person dying within 10 days of the onset of any disease—the so-called viscerotomy service (see Rickard 1937)—and the survey of different communities by the mouse-protection test (see below) to ascertain the frequency of specific serum antibodies against the yellow fever virus, showed that infection was far more widely distributed than had been realized, and that many persons must have been infected without ever developing the characteristic symptoms of the disease. It was found that, besides the urban type of yellow fever, which is carried by *Aëdes ægypti*, another type existed—provisionally referred to as **jungle yellow fever**—which occurred in districts entirely free from this species of mosquito.

It is too early yet to say very much about the epidemiology of this second type of the disease, but the evidence so far suggests that infection is maintained by certain species of tree-dwelling mosquitoes. *Hæmagogus capricorni* and *Aëdes leucocelaneus*, for example, have been found to harbour yellow fever virus (Shannon *et al.* 1938). *Hæmagogus* lives in the tree-tops during dry weather and is apparently able in rainy seasons to carry over infection from one year to the next. This probably explains why the disease is particularly liable to attack woodcutters (see Report 1940, 1941). It is known that several wild animals, including representatives of the primates, marsupials, edentates, and rodents, are susceptible to infection and not infrequently contain immune bodies in their blood. But the virus is present in the circulating blood for such a short time and immune bodies appear so rapidly that these animals cannot be regarded as affording a reservoir of infection in the strict sense. The real reservoir is the mosquito; the animals serve merely

as a convenient method of conveying infection from one mosquito to another (Bugher *et al.* 1944)

The disease is very restricted in its distribution by climate, occurring almost entirely within the tropics of Cancer and Capricorn. The endemic form occurs in both the Old World and the New. In Africa it occupies a band passing across the middle of the continent (Findlay 1941). In the New World the northern half of South America is affected. Why it has not so far invaded the Orient, where susceptible mosquitoes are abundant, is not clear. The incubation period of the disease is 3–6 days. A considerable proportion of the indigenous population of endemic areas develop only a latent or ambulant infection, after which they appear to be immune for life. Epidemics, when they occur, are never explosive, but are preceded for 2 or 3 weeks by a series of sporadic cases. They cease with the occurrence of the first frost (see Russell 1941).

Though there is reason to believe that rigid measures to diminish the prevalence of *A. ægypti* are likely to be successful in preventing the urban type of the disease, it is now clear that the eradication of infection by anti-mosquito measures alone is a hopeless task.

Animal Inoculation.—Stokes, Bauer and Hudson (1928), working in Nigeria, made a great advance when they found that Asiatic monkeys, in particular *Macacus rhesus*, could be experimentally infected with the virus of yellow fever, either by inoculation of virulent material, or by infected mosquitoes of the species *Aëdes ægypti*. Mosquitoes, when once infected, were found to be capable of transmitting the disease for the rest of their lives. It was shown that the bite of a single mosquito was sufficient to produce a fatal infection in a monkey. The virus in the circulating blood of monkeys was able to pass through Berkefeld V and N, but not through W, candles.

After subcutaneous inoculation of a *monkey* with infective material, there is an incubation period of 3 to 4 days, followed by a rise in temperature of about 3° F.; this temperature persists for 36 to 48 hours, and then falls suddenly to subnormal; collapse occurs, and the monkey dies about 5 to 6 days after inoculation. Post mortem, jaundice is usually present in some part of the body, especially the laryngeal mucosa and the subcutaneous fat; small hæmorrhages are frequently found, particularly in the lungs and alimentary canal, and in the wall of the stomach; dark coffee-ground material is often seen in the stomach and small intestine; the most constant feature, however, is the pale, mottled liver, which microscopically shows fatty degeneration and mid-zonal necrosis. The liver, when fresh, is highly infective for monkeys—in a dose of about 0·000,02 gm. When frozen, the liver remains virulent for about a fortnight; when dried and frozen, it remains virulent for about 3 months (Hindle 1929). This forms a very useful method of preserving the virus. The blood of infected monkeys contains enormous quantities of virus, proving infective sometimes in 1 : 50 – 1 : 100 million.

White *mice* are also susceptible to infection (see Theiler 1930, Dinger 1931). The intracerebral inoculation of these animals with 0·05 ml. of infective blood from a *rhesus* monkey gives rise, after an incubation period of 5 or 6 days, to an illness characterized by ruffling of the coat, inactivity, photophobia, paresis of the hind legs, tonic and clonic contractions of the whole body, and finally coma and death in 6–9 days. Post mortem there is an acute encephalitis, often with dilatation of the skin vessels, swelling of the lymph glands, hæmorrhages into the stomach, erosion of the gastric mucosa, fatty degeneration of the liver, and enlargement of the suprarenals. Infection can be carried on indefinitely by cerebral passage. According to Findlay and Clarke (1935*a*), an encephalitis may be set up in both monkeys and mice by the intranasal instillation of

a neurotropic strain of virus (see also Findlay and Stern 1935). *Guinea-pigs* can be infected intracerebrally, provided a neurotropic strain is used (Dinger *et al.* 1930, Mathis 1934, Lloyd and Mahaffy 1935).

Properties of Virus.—There is now no doubt that yellow fever is caused by a filtrable virus. Using gradocol membranes, Findlay and Broom (1933) have found that the virus is probably about 17–28 mμ in diameter. According to Pickels and Bauer (1940), who used the ultra-centrifuge method, it is only 12–19 mμ. Its successful cultivation in mouse embryo tissue was reported by Lloyd, Theiler and Ricci (1936). Jadin (1937) grew it on the chorio-allantoic membrane of hens' eggs; and Elmendorf and Smith (1937) simplified this procedure by direct inoculation of the virus into the chick embryo. When grown for several generations in tissue cultures, its virulence becomes modified; it no longer gives rise to a fatal disease when injected into monkeys subcutaneously, but it remains capable of killing mice inoculated intracerebrally (see Theiler and Smith 1937). Other changes can be produced by animal passage (see Sawyer *et al.* 1930, Findlay and Clarke 1935*b*, Findlay and MacCallum 1938). No antigenic differences have been recognized among strains of different origin. The virus contains a soluble antigenic material, which may be demonstrated as a precipitinogen in the serum of monkeys during the height of the illness (Hughes 1933). Survival of the virus in infected blood has already been referred to (see also Sawyer *et al.* 1929). It is killed by heat at 55° C. in 5 minutes, and rapidly dies in saline suspensions. In tissue, however, that is frozen solid, it remains alive for some weeks, and in tissue that is frozen and dried it retains its viability for years. It is readily inactivated by bile. In the liver of monkeys, less often of man, it gives rise to amorphous, acidophilic, nuclear degenerations. Though it is borne by mosquitoes, and may survive in them for the whole of their lifetime, there is no evidence to show that it can be hereditarily transmitted from one generation to the next (Philip 1929).

Diagnosis.—In the laboratory the diagnosis of yellow fever may be confirmed by intracerebral inoculation of the patient's blood during the first 3 days of the illness into mice. The mouse protection test (see below) may also be of value, if a specimen of serum can be obtained before the end of the first week and again in convalescence, in order to demonstrate the appearance of protective antibodies in the interval. The complement-fixation test may likewise be used early in convalescence. According to Lennette and Perlowagora (1943) complement-fixing bodies disappear fairly rapidly; their presence therefore is indicative of recent infection. For retrospective diagnosis, and for immunity surveys, the mouse protection test is used almost exclusively, since neutralizing antibodies are known to persist for years after an attack. Diagnosis at post mortem is made by histological examination of the liver, and confirmed if necessary by inoculation of the liver tissue intracerebrally into mice.

Prevention, Immunity and Vaccination.—In towns and even in country districts, where yellow fever is spread by *Aëdes ægypti*, the disease can be eradicated, as the workers of the Rockefeller Foundation have shown, by strict control measures designed to reduce the numbers of the mosquito vectors. *Aëdes ægypti* breeds not in ponds or pools, but in artificial water containers, where the natural enemies of the larvæ are fewest. It is a domesticated mosquito, and its control is therefore very much easier than it would otherwise be. In jungle yellow fever, however, anti-mosquito measures are impracticable and recourse has therefore to be had to vaccination of the exposed population.

The serum of convalescent patients and monkeys contains antibodies to the yellow fever virus, which may be demonstrated by the complement-fixation, the precipitation, or the mouse-protection test. This last test, which was elaborated by Sawyer and Lloyd (1931), consists in the intraperitoneal inoculation of mice with a fixed virus together with the serum to be tested; a simultaneous injection of starch solution is made into the brain to localize the virus. If the serum contains no antibodies, the animal dies of encephalitis. These tests, alone or in combination, have now been widely used in the survey of indigenous populations, and their results, as already mentioned, have shown the widespread distribution of infection in South America and West Africa (see Hughes and Sawyer 1932, Soper *et al.* 1932, Mahaffy *et al.* 1933, Beeuwkes and Mahaffy 1934).

The difficulty of eradicating infection completely by anti-mosquito measures has rendered some form of *vaccination* desirable for persons proceeding to endemic areas and for non-immune residents in endemic areas. Starting with the attempts of Hindle (1929), who used phenolized or formolized infected monkey liver and spleen, numerous vaccines have been developed. Since killed vaccines have met with no success (see Findlay and Mackenzie 1936), efforts have been made to find a strain of modified virulence that can be inoculated into human beings without danger. Sawyer, Kitchen and Lloyd (1932) introduced combined active and passive immunization, using a strain that had undergone variation as the result of repeated passage through the mouse's brain, together with human serum. An attenuated mouse passage virus, without serum, was recommended by Sellards and Laigret (1932, 1936); but though it has been used extensively on African natives, it has been generally regarded as too dangerous for human use. Later, a variant strain, 17D, was developed by American workers, which had been grown in tissue culture containing first mouse embryonic tissue, then whole chick embryo, and finally chick embryo from which the brain and cord had been removed. This strain was found to be greatly attenuated, failing to kill monkeys inoculated either subcutaneously or intracerebrally, and producing fatal encephalitis in mice after an average incubation period of 8 instead of 6 days; it still, however, retained its antigenic potency. Large quantities of vaccine could be prepared by inoculation of the variant strain into the developing chick embryo, incubating the eggs for 3–4 days, grinding up the embryo, suspending it in human serum, and clearing, freezing and drying the resulting mixture. For use the dried virus was rehydrated. The dosage for man was calculated according to the number of mice dying after intracerebral inoculation (Theiler and Smith 1937, Smith *et al.* 1938, Bugher and Smith 1944). This vaccine has been widely used, with excellent results. One drawback to it, however, is that occasional batches have given rise to jaundice, coming on, as a rule, 6 weeks to 4 months after inoculation (see Fox *et al.* 1942*b*). During the second world war, no fewer than 28,585 cases of jaundice occurred among the U.S. Army personnel between January and July, 1942, out of about 2½ million troops inoculated; 62 of these cases proved fatal (Report 1942*a*, *b*). There is little doubt that the jaundice was due, not to the yellow fever virus, but to the human serum used in the preparation of the vaccine (see p. 1794). Hargett, Burruss and Donovan (1943) have now prepared a similar vaccine from which the serum has been omitted. No jaundice has so far followed the use of this " aqueous base " vaccine; though Fox and his colleagues (1942*a*) have recorded a number of encephalitic reactions among those inoculated, which they attribute to variation in the 17D strain itself. The immunity conferred by the 17D chick

embryo vaccine lasts for at least 4 years and probably longer (Fox and Cabral 1943). The danger that vaccinated persons may serve to infect mosquitoes seems to be negligible, partly because the concentration of virus in the blood is very low, and partly because mosquitoes artificially infected with modified viruses of this type are unable to transmit the disease to susceptible animals (Roubaud *et al.* 1937, Smith *et al.* 1938, Whitman 1939). For an account of the ætiology, epidemiology and prevention of yellow fever, the reader is referred to articles by Sellards (1941), Russell (1941) and Gordon (1941).

Dengue Fever

Dengue fever is a disease of warm climates, and is peculiar to man; it occurs during the summer months, often in severe epidemic form, attacking a high proportion of the exposed population, and it is followed by a comparatively short-lived immunity. It is characterized by fever—usually of sudden onset and often of the saddle-back type—conjunctival congestion, pains, in the joints and muscles, leucopenia, and sometimes a rash. The disease lasts a week or ten days, and is seldom fatal. Ashburn and Craig (1907), working in the Philippines, brought evidence to show that infection was due to a filtrable virus carried by mosquitoes. By intravenous inoculation of volunteers with 20 minims of blood taken from dengue patients, they were able to reproduce the typical disease; the incubation period was as a rule 3 to 4 days—a little shorter than that of the natural disease, which is usually 4 to 8 days. Inoculation of two volunteers with blood filtered through a diatomaceous candle likewise reproduced the disease successfully. One volunteer out of four subjected to experiment developed the disease after being bitten by *Culex fatigans* Wied.

Cleland and Bradley (1918) in Australia found that the mosquito *Aëdes ægypti* was capable of transmitting infection. This observation was confirmed by Siler, Hall and Hitchens (1925) in the Philippines, who found that the patient was infective for the mosquito during the first 3 days of the disease, and that the mosquito did not become infective for human beings till the 11th day after biting. Once infected, the mosquito apparently remained so for the rest of its life. There is, however, no hereditary transmission of infection to the offspring. The mosquito, *Aëdes albopictus*, which is widely disseminated in the Far East, is also said to serve as a vector (Simmons *et al.* 1930). Observations in Greece showed that the disease could be transmitted by direct inoculation of filtered or unfiltered blood. The virus persisted in the blood for at least 5 days. The serum of patients remained virulent in sealed tubes for at least 54 days at 15°–18° C. Human volunteers inoculated subcutaneously with the ground-up bodies of *Aëdes ægypti* captured a week previously in the rooms of dengue patients, developed the disease 7–9 days later (Blanc and Caminopetros 1930, Pontano 1930). The virus passes through an L2 candle, and, according to Shortt, Rao and Swaminath (1936), can be cultivated on the chorio-allantoic membrane of the developing chick embryo.

Monkeys from non-endemic areas suffer from a very mild disease after inoculation, which is insufficiently characteristic for diagnosis. Their blood is said to be virulent for man 5–8 days, but not 12 days, after inoculation. Other laboratory animals seem to be insusceptible. In the diagnosis of dengue fever the severe leucopenia, with a proportional increase in the immature granulocytes, is of help. Second attacks of the disease are uncommon during any one epidemic. For a

general account of the disease see Simmons (1941) and the monograph by Lumley (1943).

Sandfly Fever

Synonym: Phlebotomus Fever.

This disease occurs in tropical and sub-tropical countries. It is characterized by an incubation period of 1–6 days, and a fever lasting at least 3 days, accompanied by severe headache, pains in the back, bones, joints, and muscles, and followed by considerable prostration. It is rarely fatal. Second, and even third, attacks do occur, but are not very common. The causative agent appears to be a filtrable virus. There is, however, some discrepancy of opinion on its properties. For instance, Shortt and his colleagues (1938) find that it is about 160 mμ in diameter, and that it can be grown in a tissue culture medium containing minced chick embryo, human serum and chick plasma, and on the chorio-allantoic membrane of the developing chick embryo (Shortt, Rao and Swaminath 1936). Sabin, Philip and Paul (1944), on the other hand, maintain that it is only 20–40 mμ in diameter and that it cannot be cultivated by the chorio-allantoic membrane technique. Again, according to Shortt and his colleagues (1940), the virus can be demonstrated in the patient's blood for a week in most cases, and occasionally for as long as 6 weeks. The time during which the serum is infective is given by the American observers as 3 days, namely for 1 day before to 2 days after the onset of the fever. Sera kept in the ice-chest remain virulent for months. As present in human blood, the virus, when inoculated into *rhesus* monkeys, gives rise to a mild fever; but the cultivated virus produces only a symptomless infection (Shortt *et al.* 1940). The incubation period after intracutaneous injection into human beings is said to be 2½ to 6 days (Sabin and Paul 1944). Neutralizing antibodies appear early in the serum of convalescent patients and of inoculated monkeys; they can be demonstrated by monkey protection tests, or by inhibition of the growth of the virus in culture. Immunity is said to persist for at least 4 months. Infection is spread by the sandfly *Phlebotomus papatasii* (see Whittingham 1924), though possibly other species may act as vectors. The flies do not become infective for 7–10 days after biting a patient with the fever. Diagnosis is often difficult. The absence of catarrhal symptoms is of help in distinguishing the disease from influenza, and the absence of rigors from malaria. Skin rashes do not occur (Walker and Dods 1941). The disease may closely simulate lymphocytic choriomeningitis, but the cell count in the cerebrospinal fluid is not increased (Pearson 1941). In the blood there is a leucopenia. Prophylaxis consists mainly in abolishing sandfly breeding grounds and in sleeping under a close-mesh net. Dimethyl phthalate and a pyrethrum vanishing cream are said to afford considerable protection against sandfly bites (Sabin and Paul 1944). Attempts at vaccination with living cultured virus have yielded inconclusive results (Shortt *et al.* 1940), but the use of a virus irradiated with ultra-violet light appears to be more promising (Sabin and Paul 1944).

Rift Valley Fever. Enzootic Hepatitis

In 1931 Daubney and Hudson described a new virus disease affecting sheep, cattle, goats, and man in the Rift Valley of Kenya Colony. In very young lambs the disease causes a mortality of 90 per cent. or over; but in ewes and cows the mortality is only 10–20 per cent. In man the disease resembles dengue fever,

and is characterized by malaise, nausea, rigors, abdominal discomfort, violent headache, transient fever, severe pains in the back and joints, and a leucocytosis followed by a leucopenia. Fatal cases are uncommon. By use of the mouse-protection test, the disease has now been shown to occur not only in Kenya, but in Anglo-Egyptian Sudan, in Uganda, and in the French Sudan (Findlay *et al.* 1936). In animals examined post mortem the principal finding is focal necrosis in the liver. Acidophilic intranuclear inclusion bodies can be demonstrated in the liver cells (Findlay 1933). The mode of spread of the disease is still in doubt. Daubney and Hudson (1933) bring some evidence to show that infection is carried by mosquitoes, but it is possible that it can also gain access by the respiratory tract (Francis and Magill 1935). Laboratory workers are not infrequently attacked.

Experimentally the disease can be transmitted to susceptible animals, including mice, monkeys and ferrets, but not guinea-pigs or rabbits, by cutaneous, subcutaneous, or intranasal inoculation. Mice are peculiarly susceptible, most of the animals dying in 36–96 hours after inoculation (Findlay 1932). By continued passage through the mouse's brain a neurotropic variant of the virus has been developed (Mackenzie *et al.* 1936).

The virus is widely distributed in the body, and can pass through the placenta to the fœtal organs. Its probable size is 23–35 mμ (Broom and Findlay 1933). In blood it is destroyed by exposure to a temperature of 56° C. for 40 minutes, but it survives contact with 0·5 per cent. phenol at 4° C. for 6 months. It can be cultivated in a minced chick embryo medium (Mackenzie 1933). An attack of the disease confers a high degree of immunity in sheep, and the serum of convalescent animals and human beings contains strong neutralizing and complement-fixing antibodies. Mice can be successfully immunized by a virus treated with formol or inactivated photodynamically with methylene blue (Mackenzie 1935).

An *epizootic hepato-enteritis* caused by a virus has been described in guinea-pigs (see Morcos 1933).

Bornholm Disease

Synonyms: Epidemic Myalgia, Epidemic Pleurodynia.

Though this disease has not yet been shown to be caused by a virus, a brief description of it may be given here. It is characterized by acute pain, usually of sudden onset, affecting the chest, upper abdomen, or lower region of the back on one or both sides, fever, often headache, little or no constitutional disturbance, an absence of catarrh, and a tendency to relapse. The pain is paroxysmal, and lasts for a day or more. The muscles are tender on pressure. Relapses are common at intervals of 2 or 3 days, or after a longer period. No characteristic changes occur in the blood. Fatal cases are rare. The incubation period is 2–4 days. The disease occurs in epidemic form, usually in the late summer. Infection spreads probably by contact. The disease has been observed in various parts of the world. The Danish physician Sylvest (1934), who studied an outbreak in Bornholm in 1930, has written a monograph on the disease, to which reference may be made.

Epidemic myalgia of the neck and shoulders has been described by various observers (see Beeson and Scott 1942). It differs from the Bornholm disease in not affecting the respiratory muscles of the chest and abdomen, and in being accompanied by little or no fever. Its duration is usually less than 5 days. Recurrent attacks are not uncommon, and in occasional cases the disease becomes chronic

and may pass into generalized fibrositis. Beeson and Scott have apparently been able to transmit the disease to healthy human volunteers by intravenous inoculation with blood taken from patients on the second day of the illness. The incubation period of the experimental disease was 2–4 days.

Durand's Disease

Durand (1940) described a febrile disease in himself, which he showed to be caused by a virus. Findlay (1942), who likewise contracted the disease while working with the virus in the laboratory, suggested the name Durand's disease. The symptomatology appears to be variable. Fever lasts about 10 days, and is accompanied by headache, and sometimes by symptoms of meningeal, upper respiratory or gastric inflammation. Blood taken during the fever is infective for guinea-pigs; occasionally the urine or cerebrospinal fluid may also be shown to contain the virus. Guinea-pigs are susceptible to inoculation by practically any route. After subcutaneous injection there is an incubation period of about 6–10 days, which after one or two passages of the virus falls to 2–5 days. There is fever lasting 6–10 days, local enlargement of the lymph nodes, dyspnœa, severe loss of weight, and a leucopenia after a preliminary leucocytosis. Only about 10 per cent. of the animals die—usually after 3–4 weeks. Animals killed a week or so after the onset of the fever show emaciation, a local hæmorrhagic gelatinous œdema, great enlargement of the spleen, some degree of interstitial pneumonia, and sometimes a brownish-yellow discoloration of the liver. The virus is present in the blood and organs. Intratesticular inoculation of guinea-pigs produces an orchitis. Animals that recover from the disease are immune. Cats, dogs, and *rhesus* monkeys show some meningeal reaction after intracerebral inoculation, but most other animals develop only a latent infection. Two human patients inoculated subcutaneously by Durand for purposes of pyrotherapy became febrile in 4 days; fever lasted 8 days. There were few symptoms, other than a meningeal reaction in one of the patients. The virus is estimated to be 38–57 mμ in diameter. It can be grown in serum Tyrode chick embryo tissue culture medium, and on the chorio-allantoic membrane of the developing chick embryo. In infected spleen it remains virulent for about 18 days at 4° C., and when frozen and dried for about a year. It is killed by exposure to a temperature of 65° C. for 30 minutes. No cross-immunity can be demonstrated between Durand's disease and either lymphogranuloma or lymphocytic choriomeningitis.

Distemper

Distemper is an acute infectious disease of young carnivorous animals. It is very common in puppies, and has given rise to great trouble in foxes reared for the fur industry. In the dog the disease is characterized by an incubation period of 3–6 days, an initial coryza, fever of a peculiar type, severe gastro-intestinal disturbance, and catarrhal inflammation of the respiratory tract often passing on to pneumonia. Encephalitis is a less common complication. The disease normally lasts 2–4 weeks and has a low mortality; but complicated cases, which probably constitute 40 or 50 per cent. of all cases, may last for several weeks and are accompanied by a mortality running up to 80 per cent. The disease is said to be characterized by acute anæmia and hypoglycæmia (Wharton and Wharton 1934). The encephalomyelitis that is sometimes observed is regarded by Perdrau and Pugh (1930) as being caused by a virus different from the distemper virus,

mainly because of the demyelination that is present (see p. 1921). Verlinde (1939), however, recognizes two types of encephalitis, one caused by the distemper virus itself, and another by a specifically neurotropic virus, which may cause encephalitis in the absence of distemper. The disease is spread by contact with infective secretions and discharges.

Distemper occurs naturally in the ferret, and is characterized by an incubation period of about 10 days, an initial coryza, and the formation of vesicles and pustules around the mouth ; just as in dogs, encephalitis may develop in a small proportion of cases. Distemper in the ferret is a very fatal disease, causing a mortality of about 90 per cent. (Dunkin and Laidlaw 1926). The disease has been studied to a limited extent in the cat (Hindle and Findlay 1933, Dalling 1931), the fitch (Dalling 1931*b*), and the fox (Dalling 1932*a*). In cats it seems to be due to an entirely different virus from that infecting dogs, belonging to the lymphogranuloma-psittacosis group (see p. 1877).

Distemper can be experimentally reproduced in dogs or ferrets by the subcutaneous inoculation of infective material, such as blood or spleen. Carré (1905) was able to transmit the disease to young puppies by bacteria-free exudates and by bacteria-free filtrates of infective discharges. Though M'Gowan (1911) in Scotland, and Ferry (1911) and Torrey and Rahe (1913) in America, brought forward evidence to suggest that distemper was caused by *H. bronchisepticus* (see Chapter 33), the more recent work of Dunkin and Laidlaw (1926) and of Laidlaw and Dunkin (1928) leaves no doubt that the primary cause of distemper is a filtrable virus. These workers were able to transmit the disease to dogs and ferrets by infected blood and serum from which no bacteria could be cultivated, and by Mandler filtrates of ground-up ferret's spleen. The disease reproduced by these bacteriologically sterile inocula was identical with natural distemper. Laidlaw and Dunkin regard *H. bronchisepticus* as a secondary infective agent responsible for the respiratory complications of the disease ; they draw attention to the fact that in natural distemper, in which pneumonia is not uncommon, *H. bronchisepticus*, though readily cultivable from the lung, can rarely be cultivated from the blood, thus indicating that the organism is a secondary rather than a primary invader.

The virus can be grown in tissue culture. It is present in the blood of ferrets during the first 4 days of the disease, and in the blood of dogs at the height of the first febrile period. In the tissues it gives rise to cytoplasmic and intranuclear inclusion bodies, principally in the epithelial lining of the respiratory system, urinary tract, bile ducts, and ducts of the salivary and pancreatic glands (Green and Evans 1939*a*). Their histological demonstration in the bladder, nose, or trachea affords a rapid method of diagnosis (Green and Evans 1939*b*).

Vaccination.—Laidlaw and Dunkin (1928) were able to immunize ferrets and dogs by a specific formolized vaccine. They found that 2 ml. of a 20 per cent. suspension of a ferret's spleen treated with 0·1 per cent. formol, when injected subcutaneously, would protect normal ferrets against a large dose of active virus given 2 weeks later. A similar formolized vaccine made from infected dog's liver, spleen, and lymph glands, when given in a single dose to dogs, and followed by the inoculation of an attenuated living strain of virus, produced complete immunity. It is noteworthy that vaccine made from ferret's tissues had little protective action on dogs, unless multiple injections were given ; and that vaccine made from dog's tissues had practically no protective action on ferrets, even when multiple injections were given. The vaccine has been tested under field conditions and has given

very satisfactory results. It is now made from the spleen and mesenteric lymph nodes of dogs (Laidlaw and Dunkin 1932). Experience has shown that the original "wet virus," used for the second dose is unsuitable for commercial distribution, and it has been replaced by a stable dried form of ferret virus (Dalling 1931*a*, 1932*b*). Some workers prefer to give two doses of the dog vaccine at a week's interval followed by the living virus a fortnight later (Kelser 1941). Immunization with a single dose of serum-virus mixture proved unsatisfactory and was given up (Dalling 1932*b*). If, however, a fully virulent dried ferret virus is inoculated, followed 1–2 hours later by a dose of immune serum, a satisfactory immunity results. This method is extensively used in practice in order to save the veterinarian two visits. For treatment of the disease immune dog serum may be used. It is said to be of considerable value if given within 7 days of the onset of symptoms (Wright 1932). It is also useful for prophylaxis.

Feline Enteritis

A widespread highly contagious disease of cats occurs in the United States to which the name feline enteritis is often given. It has been studied by Hammon and Enders (1939) and by Syverton and Lawrence and their colleagues (Syverton *et al.* 1943, Lawrence *et al.* 1943). Though the accounts of these observers differ to some extent, there is little doubt that they were dealing with the same disease. Kittens and young cats seem to be mainly affected. The incubation period is 4–7 days. The symptomatology is irregular, but listlessness, prostration, anorexia, vomiting, and diarrhœa are often present. The temperature is raised, and nasal and ocular discharges are common. The most characteristic feature, however, is the occurrence of a profound leucopenia, amounting sometimes to almost complete absence of white cells from the circulating blood. Death usually occurs in 2–4 days. The case mortality is given as 50 per cent. At post mortem there is little macroscopic evidence of disease ; but microscopically there is severe aplasia of the cellular elements of the bone marrow, and conspicuous intranuclear inclusion bodies are found in the epithelial cells of the intestinal mucosa and the reticular cells of the spleen and lymph nodes. Infection is spread by contact with infective discharges. A virus, capable of passing through a Berkefeld W filter, and therefore probably not greater than 35 mμ in diameter, can be demonstrated by animal inoculation in the blood, organs, nasal secretion, fæces, and sometimes urine of infected animals. The disease can be reproduced experimentally by inoculation of non-immune animals by the oral, intranasal, subcutaneous, and intravenous routes. Attempts to cultivate the virus have so far failed. The virus is specific for cats, and is apparently distinct from the feline distemper virus, but further observations are required. Recovery from the disease is accompanied by the appearance of specific neutralizing and protective antibodies.

Swine Fever

Synonyms : Hog Cholera. *Schweinepest* (German). *Peste du porc* (French).

Swine fever or hog cholera is an infectious disease, peculiar to pigs, caused by a filtrable virus. It is widespread throughout the world ; a special form of the disease has been described by Montgomery (1921) in East Africa. The disease is primarily a septicæmia, but in a large proportion of cases intestinal compli-

cations develop due to *Salm. choleræ-suis* or other members of the animal paratyphoid group, or lung complications due to *Pasteurella suiseptica*. A great deal of confusion has occurred in the past with regard to this disease, which was originally believed by Salmon and T. Smith (1886, 1885-95) to be due to infection with *Salm. choleræ-suis*; but it now appears to be fairly certain that there are at least three infections which may occur singly or concurrently. (1) Swine fever; this is due to a filtrable virus. (2) Paratyphoid of pigs; this is an intestinal disease caused by *Salm. choleræ-suis*. (3) Swine plague, this is a pleuropneumonia caused by *Pasteurella suiseptica*. The confusion has resulted from the fact that in swine fever secondary invasion with *Salm. choleræ-suis* or *Past. suiseptica* is very common.

The evidence that swine fever is primarily due to a filtrable virus was first advanced by de Schweinitz and Dorset (1903), and may be summarized as follows (Uhlenhuth and Haendel 1913): (1) Filtered blood, serum, organ suspensions, bile, or urine, when inoculated in 1–2 ml. doses, are able to give rise to the disease in normal pigs after an incubation period of about 8 to 10 days. (2) The disease may be carried over by passage from animal to animal by inoculation of filtered blood. (3) Animals that have withstood the inoculation of filtered blood are henceforward immune both to natural and experimental infection. (4) Swine that have passed through an attack of the natural disease are resistant to the inoculation of filtered blood. (5) Normal swine placed in contact with experimentally infected swine develop the disease. (6) Animals injected intravenously or fed with cultures of *Salm. choleræ-suis* develop acute intestinal inflammation, similar to that occurring in swine fever, but the filtered blood of these animals is not infective to normal swine. (7) Normal animals placed in contact with animals infected with *Salm. choleræ-suis* do not contract swine fever. (8) Animals that have overcome an infection with *Salm. choleræ-suis* are not immune to swine fever. (9) *Salm. choleræ-suis* is not demonstrable in more than a variable proportion of cases of swine fever.

Natural infection in swine fever appears to occur by ingestion of infective material, especially material contaminated with the urine of sick animals (Hutyra and Marek 1926). Animals that have recovered from the disease may continue to carry infection for weeks or months, the virus apparently persisting in the button ulcers of the intestine (Gibbs 1933).

The virus is unusually resistant to inimical agencies. In filtered blood it is said to withstand a temperature of 58° C. for 2 hours, but to be destroyed by a temperature of 78° C. maintained for 1 hour. Fluid material containing the virus retains its virulence at room temperature for 10 to 14 weeks, and dried on brick or hay for at least 28 days (Slavin 1938). In serum the virus is destroyed by 2 per cent. formol in 15 days, and by 2·5 per cent. antiformin in 2 hours (Hutyra and Marek 1926). It can be grown in tissue culture, preferably containing fresh minced pig testicle, on the chorio-allantoic membrane of the developing chick embryo, and on the surface of swine serum agar if the inoculum is previously mixed with fresh testicular cells (Hecke 1932, Tenbroeck 1941).

Attempts to confer an active immunity on swine by the injection of killed virus have failed. In practice, immunity can be produced by the simultaneous injection of 20 ml. immune serum, taken from an animal that has recovered from the disease, and 1–2 ml. of defibrinated virulent blood. Animals treated by this method are immune for life. (See also Donatien and Lestoquard 1929.)

Rinderpest or Cattle Plague

Rinderpest is an acute infectious disease of cattle, characterized by an incubation period of 3 to 9 days, acute fever lasting for 4 to 7 days, inflammation of the oral and gastric mucosa often attended by necrosis and ulceration, and a mortality varying from 15 to 75 per cent. The disease is prevalent in Southern Europe, Asia, and Africa. It also attacks buffaloes (Schein 1917). The existence of chronic carriers, with diphtheritic ulcers in the abomasum, has been recorded by Gibbs (1933).

Inoculation of normal animals with tissue fluids from an infected animal reproduces the disease. At the height of the attack the blood proves virulent in a dose of 1/1,000 to 1/25,000 ml. (Schein 1917). Goats are susceptible to experimental infection, but continuous passage through these animals is said to be followed by a diminution in virulence of the virus. There is evidence that the virus is contained chiefly within the leucocytes. Thus in blood that is allowed to clot naturally the serum is avirulent; in defibrinated blood that is centrifuged the deposit alone is virulent; in blood laked with distilled water, and centrifuged, the deposit, which consists mainly of leucocytes, alone is virulent (Kolle, see Nicolle and Adil-Bey 1902; Schein 1917, Daubney 1928). Nicolle and Adil-Bey (1902) brought forward evidence to show that the virus, when present in diluted tissue fluids or suspensions, may pass through a Berkefeld N or a Chamberland F candle; the results however are inconstant—possibly because of the intracellular location of the virus. The virus has not yet been cultivated, but Minett (1923) found that it could remain alive for at least 6 days at 37° C. in citrated blood, and for at least 13 days at 39°–40° C. in a glucose ox blood peptone water medium layered with paraffin.

Cattle may be immunized against the disease by the simultaneous injection of immune serum and living virus. In buffaloes this method is unsatisfactory, unless a large amount of serum combined with the least possible amount of virus is used (Schein 1917). Daubney (1928) states that formolized vaccines prepared from spleen pulp confer a high degree of immunity upon inoculated animals, but field inoculations with these vaccines have not yet been carried out. Pfaff (1938) recommends a vaccine made from dried goat spleen. Immunity is said to be established within 48 hours. This method has been used extensively on cattle and buffaloes, with promising results.

African Horse Sickness

African horse sickness is a highly infectious disease of horses and mules, characterized in the acute form by fever, general symptoms of illness, and pulmonary œdema; in the subacute form by fever, constitutional symptoms, and the development of subcutaneous œdematous swellings around the orbits, and not infrequently on the forehead, head, chest, abdomen, back, and extremities. The incubation period is about 6 to 7 days. In South Africa the disease occurs annually during the rainy, warmer months, January to March, especially in low moist localities, and is accompanied by a high mortality. Natural infection is probably carried by mosquitoes (Hutyra and Marek 1926). The disease can be reproduced experimentally in horses by the subcutaneous or intravenous inoculation of small quantities of blood taken from infected animals. Blood, when diluted with saline, and filtered through a Berkefeld or Chamberland candle, still proves infective. More

than one type of virus appears to exist. Prophylactic inoculation may be accomplished by Theiler's method, which consists in the injection of large quantities of immune serum intravenously, together with small quantities of virulent blood subcutaneously, followed by a second serum injection after the appearance of the febrile reaction. More recently, a specially developed neurotropic virus has been used.

Infectious Anæmia of Horses

Synonym: Swamp Fever.

This disease, which is not to be confused with the swamp fever of human beings caused by *Leptospira grippo-typhosa* (see Chapter 82), has a wide geographical distribution, occurring in various parts of Europe, the United States, Canada, Japan, and South Africa. It is most prevalent in low-lying badly drained districts, and reaches its maximum incidence during the summer months. The disease was shown by Vallée and Carré (1904) to be due to a filtrable virus, which is constantly present in the blood stream. By the gradocol membrane technique its size appears to lie between 18 and 50 $m\mu$ (Balozet 1939). Transmission probably occurs by biting flies—*Stomoxys calcitrans*. The disease can be reproduced experimentally by biting insects, but not by contact or feeding. Equines are alone susceptible; laboratory animals are refractory. The disease may be acute or chronic. The acute form is characterized by fever, extreme weakness, and conjunctival hæmorrhages, the chronic form by general unthriftiness with extreme emaciation. Anæmia does not appear to be a prominent sign. Death may occur in acute cases. Horses may remain carriers for years. There is, as yet, no specific diagnostic test or therapeutic treatment for the disease (for references see Stein 1935).

Malignant Catarrh of Cattle

Synonym: *Snotsiekte.*

This disease occurs in various parts of the world, and is characterized by fever, catarrh of the oral, nasopharyngeal, and conjunctival membranes, general enlargement of the lymphatic glands, and often symptoms suggesting involvement of the brain or cord. The mortality is subject to considerable variation. Post mortem examination of fatal cases often reveals petechial hæmorrhages in the stomach, mottling of the liver and kidneys, congestion and petechiæ in the respiratory mucosa, œdema of the lungs, enlargement of the lymphatic glands, and sometimes plate-like extravasations of blood on the surface of the brain. The disease is apparently caused by a virus, which can be transferred to rabbits and back again to cattle (see Daubney and Hudson 1936).

Ephemeral Fever of Cattle

Synonym: Three-day Sickness.

This disease is widely distributed in tropical and sub-tropical parts of the Old World. When it was introduced into Australia in 1936, it swept through the country like a pestilence. It is characterized clinically by an incubation period of 2–10 days—most commonly 3 days—sudden onset, high fever, anorexia, shivering, and frequently lameness and nasal discharge. Recovery is rapid;

relapses are uncommon ; mortality is low ; and the commonest sequela is prolonged locomotor disturbance. Blood is infective from the latter part of the incubation period to the fourth day after subsidence of the fever. The virus, which passes through a gradocol membrane of A.P.D. 0·7 μ, is associated in the blood with the leucocyte-platelet fraction. Kept at 0° C., blood remains infective for 7 weeks, and when dried and frozen for over 12 weeks. The disease can be reproduced in cattle by intravenous inoculation of infected blood. Other animals are resistant. The natural mode of spread is unknown, but sandflies are suspected. The disease leaves behind it a solid immunity. In many respects ephemeral fever of cattle resembles dengue fever in man (for references see Curasson 1936, Mackerras *et al.* 1940).

Blue-tongue of Sheep

Synonym : Catarrhal fever of sheep.

This is a specific disease affecting sheep in South Africa. It is characterized by an incubation period of about 4 days, fever lasting 5–7 days, and a catarrhal inflammation of the mucous membranes of the mouth, lips, tongue, upper air passages, and alimentary canal, and often of the laminæ and coronary band of the feet. The distinctive lesions in the mouth, which give the disease its name, do not appear for 7–10 days. The case mortality varies greatly. Blood is infective, even after passage through Berkefeld candles and after storage for 2 or 3 months under sterile conditions. The disease can be reproduced in sheep by parenteral inoculation. Infection is supposed to spread naturally by insects. Immunity can be conferred on sheep by inoculation with an attenuated virus—Theiler's vaccine (see Spreull 1913).

Nairobi Disease of Sheep and Goats

We owe most of our information on this disease to Montgomery, (1917), who studied it naturally and experimentally. It occurs enzootically among sheep and goats in the Kikuyu country, becoming epizootic when large numbers of susceptible animals are brought together. Sheep are more prone to the disease than goats, and—curiously enough—native sheep are more susceptible than pure-bred animals. The disease is characterized by an incubation period of about 2 days, a sudden rise of temperature to 105°–108° F., a fall to normal in 2 days followed by obvious illness, diarrhœa with sometimes blood and mucus in the stools, and—in fatal cases—death within 2–4 days of the onset of obvious symptoms. At post mortem there is an acute hæmorrhagic gastro-enteritis, the spleen is enlarged, the gall bladder is distended with bile, and the trachea is congested and may contain blood-stained mucus. The case mortality in native sheep is about 70 per cent. The blood is infective during the fever and for a day after its fall. The infectivity is retained after passage through a Chamberland F candle. Though the urine may also contain the virus, infection does not appear to spread by contact, but is transmitted by the bite of the " brown " tick, *Rhipicephalus appendiculatus*. Ticks can be infected in the nymphal as well as the adult stage, and there is some evidence to suggest that hereditary transmission of the infection may occur. Immunity to the disease persists for, at any rate, some months. No method of artificial immunization has yet proved satisfactory. The " brown " tick is also responsible for carrying East Coast fever in cattle. Dipping cattle

at a 3-day interval will help to clear the ground of ticks, and so diminish the risk to sheep of infection with the virus of the Nairobi disease.

Epizootic Abortion of Mares

In addition to infection with *Salm. abortus-equi* or with streptococci, a form of epizootic abortion of mares has been described that appears to be caused by a virus. The disease is very contagious. Abortion occurs, after profuse sweating but no other clinical symptoms, at the 9th to 11th month. There is a slight vaginal discharge of short duration, with rapid and complete recovery of the mare. Dimock and Edwards (1936) observed several outbreaks in the United States. No bacterial cause could be found. In many of the aborted fœtuses there was a clear yellow or reddish-yellow exudate in the pleural cavity, numerous small white foci of degeneration in the liver just beneath the capsule, congestion of the mesenteric and colonic lymph nodes, and petechial and lineal hæmorrhages in the heart. Pregnant mares, fed on fluids and tissues from infected fœtuses, either before or after Seitz filtration, aborted in 18 to 34 days. Dimock and Edwards obtained doubtful results in their attempted transmission of the disease to guinea-pigs, but Miessner and Harms (1937) in Germany were apparently more successful. By subcutaneous and intraperitoneal inoculation of filtered material from the liver, spleen, kidneys, and stomach contents of aborted fœtuses, they were able to reproduce the disease in pregnant guinea-pigs, and to carry on infection through 4 passages.

Fowl Plague or Fowl Pest

This is an acute infectious disease of fowls, and exceptionally of geese, which was studied by Landsteiner (1906) and Russ (1906). It does not appear to spread by contact, and the natural mode of infection is unknown. The incubation period is 3–5 days; the symptoms consist of depression and dullness, darkening of the comb and wattles, and inflammation of the buccal mucosa. The disease lasts 2–4 days, and is very frequently fatal (Hutyra and Marek 1926). The experimental disease may be produced in chickens by the inoculation of blood, nasal secretion, or cloacal secretion in minimal quantities. Pigeons are resistant. The minimal infecting dose of fowls' blood is seldom above 0·000,000,1 ml. Ferrets and *rhesus* monkeys are said to show some degree of susceptibility (Findlay and Mackenzie 1937). The virus is able to pass through porcelain candles, and according to Elford and Todd (1933) is 60–90 mμ in diameter. Todd (1928*a*) finds that in the centrifuged blood of infected fowls the virus is contained chiefly in the leucocytic layer, the concentration in this layer being roughly 100 times that in the clear plasma or washed red blood corpuscles. Doerr and Gold (1932), however, disagree with this, and bring evidence to show that the virus is adsorbed on to the red cells (see also Doerr and Seidenberg 1932). Attempts to cultivate the virus in defibrinated fowl blood have been unsuccessful, but Burnet and Ferry (1934) have grown it on the chorio-allantoic membrane of the developing chick embryo. In contrast to the Newcastle disease virus (see below), no focal lesions are found in egg cultures. Immunization experiments have been disappointing. Todd (1928*b*) was able to protect fowls against at least 300,000 lethal doses by giving three injections at weekly intervals of a phenolized vaccine, but his results still await confirmation. Heated or formolized vaccines both proved unsatisfactory.

Whether the plague affecting birds of the *thrush* family, described by Maggiora and Valenti (1903), is related to fowl plague is uncertain.

Newcastle Disease of Fowls

This disease, which was described by Doyle in 1927, was first observed on a poultry farm near Newcastle-on-Tyne, but has since been reported from many parts of the world. It is an acute, febrile, contagious disease of fowls, which resembles fowl plague, but differs from it in a number of particulars. In addition to general symptoms of sickness, there is an offensive watery diarrhœa, a mucous discharge from the nostrils and mouth, and, most characteristic of all, difficulty in respiration manifested by the long gasping inhalation through the half-opened beak. The incubation period is 4–11 days. The morbidity in infected flocks is very high, and the case mortality verges on 100 per cent. The disease can be readily transmitted not only to fowls, but also to pigeons, which it will be remembered are insusceptible to fowl plague. The causative agent is a virus, which, according to Burnet and Ferry (1931), is 80–120 mμ in diameter, and can be readily cultivated on the chorio-allantoic membrane of the developing chick embryo. The virus kills the embryo in 24–72 hours; post mortem, hæmorrhagic encephalitis and hæmorrhagic lesions, particularly in the developing feather follicles, are striking features (Burnet 1942). Inoculated by the allantoic route, it multiplies abundantly, and the allantoic fluid gives the Hirst agglutination reaction with chicken red blood corpuscles. After intranasal inoculation into mice, it produces consolidation of the lungs. In these and some other ways the Newcastle virus closely resembles the influenza virus of man. It is not, however, pathogenic for ferrets, nor does it appear to be antigenically related to the virus of influenza (Burnet 1942), though it behaves like it in many important respects and has in fact been responsible for one case of human infection (Burnet 1943). It is found in the body fluids, organs, and excretions of affected birds. Cross-immunization tests show that the fowl plague and Newcastle viruses are distinct.

Infectious Laryngo-tracheitis of Fowls

This disease has so far been reported mainly from North America and Australia. It appears to have been first recognized and studied in the United States by May and Tittsler (1925), Beach (1931), and others. Hyperacute, acute, chronic and asymptomatic forms are recognized clinically (Seddon and Hart 1935). Affected birds suffer from difficulty in respiration. The head is elevated, the neck extended, and air is drawn in through the half-opened beak with a loud wheezing sound. During expiration the head is lowered, and violent fits of coughing may occur, accompanied by the expulsion of masses of clotted blood and mucus. Fowls of all ages are susceptible. The incubation period is 7–12 days. The morbidity in an infected herd is 50–90 per cent. The case mortality varies in different outbreaks from 5–90 per cent. averaging about 20 per cent. (Doyle 1933). The disease is contagious, and there is evidence that chronic carriers may play a part in keeping infection alive from one outbreak to the next (Gibbs 1933). Intratracheal inoculation of infective material into normal fowls readily sets up the disease, but pigeons are resistant (Seddon and Hart 1936). The causative agent appears to be a virus, which is said by Gibbs (1935) to pass through a membrane with an average pore diameter of 0·082 μ. This means that the virus is smaller than that of fowl plague or the Newcastle disease. Burnet (1936) has cultivated the virus on the chorio-allantoic membrane of the chick embryo, on which under suitable conditions it gives rise to pocks. By counting the number of pocks developing after inoculation

with mixtures of virus and immune serum, he has been able to study the process of serum neutralization. The results suggest that epizootic strains are more readily neutralizable by serum than enzootic strains, even though no qualitative antigenic difference may be detectable between the two strains. According to Brandly (1937) the fowl-pox virus can develop on the chorio-allantoic membrane of the eggs of the chicken, turkey, duck, guinea-fowl, and pigeon, whereas the laryngo-tracheitis virus will grow in the chicken and turkey egg, but not in the duck, guinea-fowl, or pigeon egg. Fowls may be actively immunized against the disease by inoculation of virulent material into the cloaca and bursa of Fabricius. The resulting lesions are not serious, and leave behind them a substantial degree of immunity (Beaudette and Hudson 1933). The best time to vaccinate is between the age of 16 and 20 weeks (Gibbs 1936). For the differential diagnosis of the disease from fowl-pox, coryza, and A-avitaminosis, the reader is referred to Seddon and Hart (1935).

Kligler and Perek (1942) have described a disease among chicks in Palestine, resembling infectious laryngo-tracheitis but differing from it in its mildness. The disease spreads readily by contact, and can be reproduced experimentally by intratracheal or intraperitoneal inoculation with a Seitz filtrate of lung or tracheal mucus. The virus is present on the surface of the eggshell, where it can be destroyed by permanganate.

Infectious Bronchitis of Chickens

Beaudette and Hudson (1937) have described an epizootic disease which they think has probably been confused in the past with infectious laryngo-tracheitis. It affects young chicks—usually less than a fortnight old—is characterized by symptoms similar to those of laryngo-tracheitis, and is accompanied by a high mortality. At post mortem, the bronchi contain thick mucus or cheesy plugs, the abdominal air-sacs are turbid, and there is a fibrinous exudate in the pericardial cavity. The virus passes a Berkefeld W filter. It produces the disease on intratracheal or intranasal inoculation into young chicks, but not with regularity on subcutaneous or intramuscular inoculation. After a few passages, it grows well on the chorio-allantoic membrane of the developing chick embryo, but unlike the virus of Newcastle disease or of laryngo-tracheitis, it gives rise to no focal lesions; the embryo, however, is killed. Serum from convalescent birds contains neutralizing antibodies. There is no cross-immunity between infectious bronchitis and laryngo-tracheitis.

Blue Comb of Chickens

Synonyms: Pullet disease, X Disease.

This disease has been observed in the north Atlantic and north central area of the United States. It affects pullets mainly 5–7 months of age, though chicks only 4 weeks old may be attacked. Turkeys may also suffer. The incidence is highest in the summer months. The morbidity and mortality in a flock are very variable. According to Jungherr and Levine (1941), who described the disease, the morbidity varies from 0·1 to 100 per cent. with a mean of about 20 per cent., and the case mortality from 0·2 to 53 per cent. with a mean of about 5 per cent. The disease is characterized by a fall in egg production and food consumption, darkening of the comb, droopiness, cyanosis, dehydration, diarrhœa or constipation,

and occasionally pyrexia. Pathologically, the chief lesions are focal necrosis of the liver, serous petechiæ, enlargement of the pancreatic islands, patchy muscular degeneration, and nephrotic changes in the renal glomeruli and tubules. Jungherr and Levine regarded the disease as an endogenous toxæmia, but Waller (1942) has brought evidence in favour of a virus origin. From the blood of acutely affected birds he has isolated a filtrable agent that grows readily on the chorio-allantoic membrane of the developing chick embryo. Susceptible chickens inoculated with infected membrane become depressed and cyanotic in 3–4 days. They do not die but, if killed after 4–5 days, they show subcutaneous œdema, generalized jaundice, hæmorrhages into the skeletal muscles, petechial hæmorrhages in other organs, congestion and swelling of the liver and kidneys, and acute catarrhal hæmorrhagic duodenitis. Fæces of experimentally infected birds are infective to other birds by the mouth. Intraperitoneal inoculation of susceptible chickens with a bacteria-free filtrate of infected fæces likewise reproduces the disease. The virus passes through a Chamberland L3 and a Seitz filter. Its relationship to the disease must await further inquiry.

Virus Disease of Owls

A virus disease affecting the great horned owl (*Bubo virginianus*), and transmissible experimentally to the same species and to the screech owl (*Otus asio*), has been recognized by Green and Shillinger (1936) in the United States. The disease is characterized by the formation of multiple abscesses in the liver, and, less constantly, in the spleen. Large intranuclear inclusion bodies occur in the hepatic cells. Nothing is known about other properties of the virus.

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CHAPTER 89

FILTRABLE VIRUS DISEASES—*Continued*

E. GROUP CHARACTERIZED BY TUMOUR FORMATION

THE group of virus diseases characterized by tumour formation is of particular interest in affording a possible clue to the genesis of neoplasms. In this chapter we must confine ourselves to factual description; but those who are interested in the wider aspects of the subject would do well to refer to the far-reaching speculations of Andrewes (1939*b*) and of Rous (1943).

FOWL LEUCÆMIA

This is an infectious disease of the blood-forming organs, associated with atrophy of the bone marrow and changes in the viscera. The disease may be transmitted to normal fowls, but not to other birds, by intravenous inoculation with the citrated blood of infected fowls. According to Ellermann (1921), the virus can pass through a Berkefeld candle. Ellermann found that one strain of virus gave rise to three different types of leucæmia: (1) a myeloid type; (2) a lymphatic type; (3) an intravascular lymphoid type, characterized by intravascular deposits of lymphoid cells. An increase of virulence was noted during its passage through fowls, shown by a shortening of the interval between inoculation and death, from 15 to 20 weeks at the commencement, to 6 to 8 weeks at the end of the series of experiments. In spite, however, of the increased rate of killing, the infecting power of the virus, as judged by the proportion of successful inoculations, remained approximately constant at 20–40 per cent. Ellermann was unable to produce active immunity to the disease by subcutaneous inoculation of virulent material.

Furth (1932) found that infection could be transmitted by 0·000,001 ml. of cell-free plasma, and that the virus could withstand drying for at least 54 days. He also (1933) described a new transmissible strain of chicken leucosis which gave rise to (1) lymphomatosis with or without tumour formation, (2) myelocytomatosis with or without leucæmia, (3) endothelioma. The histology of the disease is beautifully illustrated in the monograph by Andersen and Bang (1928).

FOWL PARALYSIS

SYNONYMS: Lymphomatosis: neurolymphomatosis.

Though there is as yet no proof that this disease is caused by a virus, it may be well to make a brief mention of it here. The disease is common in both Europe and America. It affects particularly young birds, but may occur at any age. In many flocks it appears year after year. Some strains of birds appear to be more susceptible than others. The symptoms consist of asymmetrical paralysis

of the wings and legs, usually of slow onset, and more often spastic than flaccid. There is general loss of condition, and sometimes blindness. The disease is usually fatal, but recovery may occur. Pathologically, there is intense mononuclear cell infiltration of the nerves, especially the brachial and sciatic, leading to partial degeneration of the nerve fibres. The posterior root ganglia and, to a less extent, the spinal cord may be affected. Lymphomatous deposits, amounting at times to tumours, may be present in the viscera, especially the ovary. There is no leucæmia. The disease can be reproduced irregularly in normal birds by the intraperitoneal or intracerebral injection of unfiltered lymphomatous tissue. About 25 per cent. of the inoculated birds may develop symptoms, after an incubation period of 2–4 months, but the rest remain healthy. By inbreeding, a strain of fowls may be obtained that are highly susceptible to natural or experimental infection. Passage of the virus through these birds results in an increase in its virulence with the production of an acute disease accompanied by inflammatory lesions in the heart and liver, and later by lymphomata. The evidence suggests that fowl paralysis is a chronic form of a disease that is partly or wholly symptomless during the acute stage. Attempts to demonstrate a virus by culture on the chorio-allantoic membrane of the developing chick embryo have yielded inconclusive results (see Pappenheimer *et al.* 1929*a*, *b*, Galloway 1929, McGaughey and Downie 1930, Dalling and Warrack 1936, Blakemore 1939, 1940).

Rous Sarcoma of Chickens

Rous (1911, Rous *et al.* 1912, Rous and Murphy 1914) described three transplantable sarcomata of fowls. Tumour I was a spindle-celled sarcoma that metastatized freely and caused death in a month; Tumour VII was an osteochondrosarcoma; and Tumour XVIII was a spindle-celled sarcoma with blood sinuses into which growth occurred. Each of these three tumours could be transmitted to normal fowls by the inoculation of cell-free Berkefeld filtrates. Gye (1925), who worked with the Rous Tumour I, has recorded evidence from which he concludes that the active agent is a living filtrable virus, which can be cultivated anaerobically in tubes of Hartley's broth to which 17 per cent. of rabbit serum, 0·2 per cent. KCl, and a fragment of chick embryo have been added. He believes that the tumour-forming activity of this virus is dependent on the presence of another, non-living factor, which determines the type of cell which is infected, and hence the type of tumour which is produced. This second, auxiliary agent he calls the "specific factor." The evidence in favour of the dual nature of the active agent in tumour-filtrates consists in the differential action of aerobic incubation and of various chemical agents. In an extensive series of experiments Gye found that (*a*) primary "cultures" incubated aerobically at 37° C. for 3 days were inactive; (*b*) filtrates, obtained by passing the ground-up tumour through a mixture of paper pulp and sand, when treated with chloroform, were inactive; but that (*c*) the inactivated "cultures" and the chloroform-inactivated filtrates, when acting in combination, gave rise to typical tumours. He would regard the specific factor in the "cultures" as being destroyed by aerobic incubation, and the virus in the tumour filtrate as being destroyed by the chloroform. He would also extend the conclusions based on these results to malignant neoplasms in general. Gye's results have not yet been completely confirmed by other workers, and his hypothesis is still in the stage of discussion and experiment (Gye and Mueller 1929, Amies and Carr 1939).

During the past two decades fresh filtrable tumours of fowls and other birds have been described, and considerable interest has centred around their ætiology. McIntosh (1933) and McIntosh and Selbie (1939) have reported that tumours induced by tar may be transmitted to normal birds by cell-free filtrates; and Selbie and McIntosh (1939) have found that the filtrability of fowl tumours may be increased by such procedures as proteolytic digestion or freezing and thawing, which presumably act by liberation of virus from the cells in which it is growing. The demonstration by Ledingham and Gye (1935) of what appear to be elementary bodies in infective filtrates, and the immunological studies of Andrewes (1931, 1932, 1933, 1936, 1939*a*), suggest more and more strongly that viruses, not differing in any important respect from those known to give rise to ordinary infectious diseases, play an essential part in the causation of these tumours (see Andrewes 1934). The particle size of the Rous sarcoma virus has been determined by Elford and Andrewes (1935, 1936). By the gradocol membrane technique it appears to be 75–150 mμ in diameter, and by the centrifugation method 60–70 mμ.

Molluscum Contagiosum

This is a contagious skin disease of man characterized by the formation of small red masses, which later take on a warty appearance, undergo necrosis, and discharge caseous material. MacCallum (1892) described peculiar bodies in material taken from the disease, which he regarded as due to nucleolar extrusion. They were later described by Lipschütz (1907) as very small rounded bodies, about 0·25 μ in diameter, arranged singly or less often in pairs. They can be stained with Loeffler's flagellar stain or with Giemsa. These molluscum bodies were at one time considered to be parasites, but are now generally regarded as inclusion bodies (Goodpasture and King 1927). The disease is apparently caused by a filtrable virus, since positive inoculation experiments in man have been reported with Chamberland filtrates of the tumour (Juliusberg 1905).

Common Warts

Numerous workers have recorded evidence which suggests that common warts are infectious. Jadassohn (1896) found that they could be produced in human beings by experimental inoculation. More recently Wile and Kingery (1919), and Kingery (1921), in a small series of experiments, were able to produce warts by intracutaneous inoculation of human beings with a Berkefeld filtrate of a suspension of ground-up wart material. In one case the warts were carried over successfully for two generations. The incubation period varied from 4 weeks to 6 months. Venereal warts—*condyloma acuminatum*—are infective and are apparently caused by a virus (see Findlay 1930); occasionally they may become cancerous. Warts are not uncommon in animals. According to Findlay (1930), there is some evidence to suggest that they are species-specific. For example, dog warts failed to infect man, rabbits, mice, or guinea-pigs; and human warts were non-infective for dogs, rabbits, guinea-pigs, rats, or mice.

Infectious Myxomatosis of Rabbits

Sanarelli (1898) described an infectious myxoma indigenous to rabbits of South America. The disease can be conveyed by inoculation of filtered material. Tumour masses appear quickly at the site of inoculation, and later at various other points in the subcutaneous tissue, as well as in the lymph glands and spleen. The disease

is very malignant and kills rabbits in 7 to 15 days. Rivers (1926–27) finds that both epithelial and connective tissue are affected, the epithelial cells being destroyed, and certain of the connective-tissue cells undergoing proliferation. In the epidermal cells he describes small pink granular areas occupying the cytoplasm. They increase rapidly in size and form acidophilic masses often containing blue, round or rod-shaped, bodies. Lipschütz (1927), who has made similar observations, regards these round or rod-shaped bodies as micro-organisms.

The virus is widely distributed throughout the tissues, though most abundant in the swollen tissues and lymph glands. It is killed by heat at 50° C. in 15 minutes. It is quickly inactivated by a pH of less than 4·6. It withstands the action of glycerol for a year in the ice-chest. It is highly species-specific, and appears to be almost uniformly fatal for the domestic rabbit (see Rivers 1930, Findlay 1933, Hyde and Gardner 1933, Ledingham 1937). Relatively pure elementary body suspensions have been prepared by Rivers and Ward (1937). They have been shown to contain a soluble antigen, which may be demonstrated as a precipitinogen in the serum of infected animals. This antigen, which is a heat-labile protein, stimulates the production in rabbits of precipitins and agglutinins, but not of neutralizing antibodies (Rivers *et al.* 1939). By microscopical examination van Rooyen (1937*b*) estimates the elementary bodies to be about 0·31–0·36 μ in diameter, but other methods suggest a size of only 125–225 mμ (see Mueller 1941, Stanley 1943). The virus has been cultivated on the chorio-allantoic membrane of the developing chick embryo (Lush 1937, Hoffstadt *et al.* 1941). The relation of this virus to the virus of infectious fibromatosis of rabbits will be discussed below. A neurotropic strain of the myxoma virus has been developed by Hurst (1937) as the result of serial passage through the brain of rabbits. This strain does not prove fatal on intracutaneous inoculation, and the histological picture to which it gives rise differs from that of the parent strain. Animals inoculated with the neuromyxoma strain are protected against subsequent inoculation with the myxoma-producing virus.

Infectious Fibromatosis of Rabbits

This disease, which was first observed by Shope (1932) (see also Shope 1936*a*) in cotton-tail rabbits, is of the nature of a filtrable fibroma, which occurs in the subcutaneous tissue. It can be transmitted by filtrates of the tumour suspension to wild and domestic rabbits, but not to other animals. Cytoplasmic inclusions are demonstrable in the cells. The condition is non-fatal, and undergoes spontaneous retrogression. The virus is resistant to glycerol, and is apparently different from that causing infectious myxomatosis. A very remarkable mutant strain has been derived from it which gives rise to acute inflammatory lesions instead of the usual proliferative changes (Andrewes 1936, Shope 1936*b*, Andrewes and Shope 1936, Editorial 1936). Rabbits that have been inoculated intramuscularly with tar respond in an abnormal way to the infectious fibroma virus. Regression of the lesions produced by intradermal or subcutaneous inoculation of virus is much delayed, continuous tumour growth is not uncommon, and transformation to a fibrosarcoma may apparently occur (Ahlström and Andrewes 1938, Andrewes and Ahlström 1938). The diameter of the fibroma virus has been estimated by the gradocol membrane technique to be 125–175 mμ, and by the centrifugation technique 126–141 mμ (Schlesinger and Andrewes 1937).

The remarkable relationship of the infectious fibroma virus to the infectious

myxoma virus of rabbits was first pointed out by Shope (1932), who found that animals which had recovered from infection with the fibroma virus enjoyed a considerable measure of immunity to the myxoma virus. Conversely, rabbits that recover naturally from myxomatosis—and these are very few—are immune to the fibroma virus (Ledingham 1937). The mechanism of this cross-immunity is not clear, but there is little doubt that the two viruses agree closely in antigenic structure. Berry and Dedrick (1936) and Berry (1940) found that, if a mixture of myxoma virus, which had been inactivated by heating at 60°–80° C. for 30 minutes, and of active fibroma virus was injected into a rabbit, myxomatosis resulted. This observation has been confirmed by several workers (for references see Gardner and Hyde 1942), but its interpretation is at present in doubt. Whether the fibroma virus undergoes transformation into the myxoma virus, or whether the apparently killed myxoma virus is activated by the living fibroma virus, the available evidence does not permit us to say.

Infectious Papillomatosis of Rabbits

This disease has been described by Shope and Hurst (1933). It affects cottontail rabbits, and is characterized by the presence of warty or horn-like protuberances on various parts of the body. Infection can be transmitted in series through cottontail rabbits. Only a proportion, however, of these animals are susceptible, and in more than half of those animals in which papillomata do develop retrogression of the growths occurs sooner or later. Malignant change is rare. The domestic rabbit, on the other hand, is highly susceptible. Not only do papillomata develop readily, but a considerable proportion of them undergo carcinogenic transformation (Rous *et al.* 1936). According to Shope (1937), papillomatosis of the domestic rabbit cannot usually be transmitted by passage, nor is it easy to demonstrate the presence of virus in the growths (see also Kidd 1938). The papilloma virus can infect epidermal tumours induced by tar, and may bring about in them an abrupt change into carcinomata; direct demonstration of the virus in the carcinomatous tumour has so far failed, but its presence can be shown by serological methods (Rous 1943). An even more rapid method of bringing about the carcinomatous state is to apply methylcholanthrene and tar to virus-induced papillomata; the chemical agents appear to act, not as ordinary stimulants of cell proliferation, but as specific carcinogens affecting the virus (Rous and Friedewald 1944).

The papilloma virus is filtrable through a Berkefeld candle, resists the action of glycerol, and is killed by exposure to 70° C. but not 67° C. for 30 minutes. By the gradocol membrane technique its size has been computed as 23–35 mμ, and by the centrifugation technique as 32–50 mμ (Schlesinger and Andrewes 1937). Studies by the electron microscope suggest that the particles are spherical and have a mean diameter of about 44 mμ (Sharp *et al.* 1942). Rabbits with experimentally produced papillomata are partly or completely immune to re-infection, and their serum has neutralizing and complement-fixing properties (see Kidd 1938). Artificial immunization by intraperitoneal injection of glycerolated or non-infective virus has been described (Shope 1937).

Oral Papillomatosis of Rabbits

Parsons and Kidd (1943) have met with a disease among domestic rabbits in New York City characterized by the occurrence of small, usually multiple, benign

papillomata on the under side of the tongue. They persist often for several months but, unlike the Shope papilloma, do not become malignant. The disease can be reproduced in rabbits and horses, but not in other animals, by local inoculation of a tumour suspension. The resulting lesions are strictly confined to the oral mucosa—a site which is not affected by the Shope virus. Infection does not spread readily by contact. It is probably transferred from the mother to the young by suckling, and remains latent till it is activated by trauma or, experimentally, by tar. The virus passes through a Berkefeld N candle. In water-clear suspensions it is destroyed by exposure to 75° C. but not 65° C. for 30 minutes. In 50 per cent. glycerol Locke solution in the ice-chest it survives for 1–2 years at least.

Hodgkin's Disease

Little is known of the ætiology of this fatal disease, in which characteristic histological changes are usually found in the enlarged lymph glands and lymphoid deposits. Gordon (1933, 1934, 1936) has described the reproduction of meningo-encephalitis in rabbits by the intracerebral inoculation of autolysed gland suspensions. Elementary bodies are said to be distinguishable in human material and in impression preparations of infected rabbit's brain. Gordon's test is said to be of diagnostic value in about 75 per cent. of cases of Hodgkin's disease (van Rooyen 1934, 1937*a*). The encephalitogenic agent is said to pass through British Berkefeld and Seitz EK filters; to be inactivated by exposure to a temperature of 80° C. for 30 minutes; to withstand 0·5 per cent. phenol at 37° C. for probably a week or longer; and to be neutralized by the serum of a rabbit that has recovered from the infection (van Rooyen 1934). In considering the nature of this agent it is interesting to note that Friedemann and Elkeles (1933) reported the production of encephalitic symptoms in rabbits following intracerebral injection with bone marrow from cases of acute leucæmia and pernicious anæmia. Friedemann (1934) demonstrated the presence of a similar property in normal human leucocytes bone marrow, and splenic tissue. MacKenzie and van Rooyen (1935) confirmed these findings, and concluded that the agent present in certain normal tissues, is indistinguishable from that present in lymphadenomatous glands. What the nature of this agent is, it is difficult to say. Its resistance to heat, alcohol, and ether render it unlikely that it is a virus. Moreover, the inability to transmit the agent from one rabbit to another, and the susceptibility to re-inoculation of rabbits that recover, are points against its virus nature (van Rooyen 1934). Van Rooyen (1937*a*) sums up by concluding that Hodgkin's disease is essentially a neoplastic process affecting lymphatic gland tissues. Whether or not a virus is concerned in the neoplastic activity must, for the present, remain an open question.

It may be noted that Petzetakis (1936, 1937*a*, *b*) described the isolation of a so-called neurolymphophilic virus from a patient suffering from chronic suppurative lymphadenitis. Inoculated intraperitoneally into guinea-pigs, it gave rise to hypertrophy of the lymph nodes, Peyer's patches, and spleen, and inoculated intracerebrally to a fatal meningo-encephalitis. Rabbits and spermophiles were also found to be susceptible. Petzetakis' findings were not confirmed by Béquignon and Reinié (1937).

It is of interest that organisms of the *Brucella* group have been isolated from the lymph nodes of patients suffering from Hodgkin's disease (Poston and Parsons 1940); but there is no reason to suppose that they are causally related to this disease.

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CHAPTER 90

THE NORMAL FLORA OF THE HUMAN BODY

THE normal flora of the human and animal body has received relatively little attention from bacteriologists in the past, and it is only within recent years that any serious attempt has been made to study it in detail. Before describing the organisms met with in different parts of the body, it is necessary to realize certain general principles regulating their distribution. Firstly, our conception of the normal flora depends very largely on the technique that is employed. A survey based upon examination of stained smears frequently gives results quite different from a survey based on cultural examination. And the results obtained with one culture medium may differ widely from those obtained with another. The main reason for this difference is that every culture medium is more or less selective in its action; it encourages the growth of some organisms and hinders or entirely prevents the growth of others. For example, very different pictures of the intestinal flora will be obtained on agar plates and on MacConkey plates; on the latter medium the bile-salts will inhibit the growth of nearly all organisms except the Gram-negative coliform bacilli, whereas on agar numerous cocci, Gram-positive bacilli, and other organisms will develop as well. Similarly, very different pictures of the nasopharyngeal flora will be obtained if swabs from this region are plated (*a*) on ordinary agar, (*b*) on blood agar, and (*c*) on a selective medium such as Fildes' agar. The method of incubation is of great importance; cultures incubated anaerobically will reveal a very different flora from those incubated aerobically. Incubation at 22° C. is unfavourable to the growth of many pathogenic species.

Having realized the importance of the technique employed, it is necessary to understand that the term "normal" flora may be misleading. It is possible to give a description of the intestinal or nasopharyngeal flora which will apply to the great majority of adult persons living under particular environmental conditions; but this description may not be applicable, or may apply only in part, to another community, consuming a different diet, or exposed to a different climate. There are certain bacterial species which, so far as our information goes, are constantly present in particular situations. Thus, *Bact. coli* appears to be a normal intestinal parasite of man in all parts of the inhabited globe; *Str. viridans* is, so far as we know, always present in the nasopharynx; and *Staph. albus* seems always to be present on the normal skin. In addition to such constant constituents of the human bacterial flora, there are, however, very many species whose range is limited in space and time. Many of these species are potential pathogens, such, for instance, as the pneumococcus, the meningococcus, the influenza bacillus, and hæmolytic streptococci, among the nasopharyngeal flora; but it is probable that many non-pathogenic species show a similarly localized

distribution. If our knowledge were more extensive than it is, we should probably be able to describe a basal flora, characteristic of mankind under all conditions; a supplementary flora, varying in frequency, but ranging widely as normal parasites of man; and various species showing a restricted range or a temporary prevalence, conditioned by localized or transient environmental factors. This aspect of bacterial ecology has, however, received relatively little attention, except with regard to the incidence of a few important pathogenic species. If we include, among the normal flora of a particular region of the body, all those bacterial species which may be isolated from any considerable sample of apparently healthy persons, we must note that this flora varies widely, from time to time, and from place to place.

The nature of the intestinal flora, for example, depends on a number of factors, such as diet, gastric acidity, degree of peristalsis, external temperature, and so on. The predominant flora on a herbivorous differs from that on a carnivorous diet; in the upper part of the small intestine it is more copious in persons with gastric hypoacidity than in normal persons. Again, the flora of the nasopharynx is constantly changing both in the same individual and in the same community. At one time, pneumococci and Pfeiffer's bacilli may be present in large numbers in apparently healthy persons, whereas at another time they may be far less frequent. It must be realized, therefore, that, in the account which follows, we can make no attempt to give a complete description of the organisms that may be found in different parts of the body, or to indicate more than roughly their frequency and relative proportions.

The species found in the regions of the body which are normally colonized by bacteria include those generally accepted as pathogens, those generally accepted as saprophytes, and many that are intermediate. We know very little of the nature of the association between the bacterium and the healthy host tissue in any of these three cases. We may regard all typical members of a pathogenic species as virulent, and postulate a high level of immunity, perhaps specific, in order to explain the prevalence of the organism in the normal flora; or we may postulate a wide variation of virulence in the species, and assume a lesser virulence for the strains found in healthy tissue. For example, as we saw in Chapter 74, the "carrier" types of pneumococci appear to invade the lung tissue of carriers only when the host is subjected to debilitating influences, and the "infective" types may be carried by healthy people who have acquired an immunity to them (see, for example, Finland, Brown and Barnes 1940); and there are indications (Chapter 54) that the normal fusiform bacilli of the mouth may initiate ulcerative lesions in the mouth of vitamin-deficient animals. But except where there is an abnormally high specific antibody response to a species that occurs in the flora of normal tissues, any explanation in terms of a balance between the virulence of the "pathogen" and the resistance of the host is little more than a re-description of the observed state of affairs, though it serves to emphasize that the coexistence of bacterial with host cells is the result of an equilibrium between two sets of factors, and not merely a mutual indifference to each other's presence. And by analogy with a large number of instances of biological associations variously described as parasitism, saprophytism, commensalism, symbiosis, etc., we may expect that the associations of saprophytes and healthy tissue cells are equally dynamic.

Moreover, equilibria exist not only between host and "normal" flora, but

between the different species within the flora; and the association of various bacterial species on a given surface of the body is relevant to any discussion of infection. It cannot be too strongly emphasized that none of those regions of the body, to which bacteria can gain entrance by the normal portals, provides a virgin soil in which any new-comer may flourish. It is just as impossible to ensure the proliferation of a particular bacterial species by introducing it into the mouth, as it is to ensure a crop of a particular plant by scattering seeds in a field already occupied by a pre-existing plant-association. The new-comer will have little chance of survival, unless it is adapted to occupy some definite place in its new environment. It is fairly certain that those pathogenic bacteria which spread readily from host to host owe their capacities in this direction to their ability to colonize on skin or mucous surfaces, or to escape from the superficial environment to the underlying tissues. This question, in relation to the upper part of the respiratory and alimentary tracts, has been studied in some detail by Bloomfield and his colleagues (Bloomfield 1919, 1921, 1922*a*, *b*, *c*, *d*, *e*, Bloomfield and Felty 1923*a*, *b*, *c*, Felty and Bloomfield 1923).

Normal Flora of the Alimentary Tract.

The bacteria present in the *mouth* are subject to great variation both in number and in kind. Even the relatively clean and healthy mouth contains a considerable amount of detritus and other organic matter derived from particles of food, desquamated epithelium, pharyngeal mucus, and other sources; these provide nutriment for a diverse flora which is of necessity undergoing frequent change. Moreover, inflammatory processes of the teeth and gums are often present, favouring the growth of pyogenic bacteria. The saliva has apparently only a slight bactericidal effect, and it is perhaps surprising that, with so much organic matter in the mouth and so many organisms to grow in it, septic infections are not more common. There are probably three main reasons for this: firstly, the saliva, though not conspicuously bactericidal, by its constant secretion tends to flush the mucous surfaces of the mouth, and wash off many of the adherent organisms, which, when swallowed, are rapidly killed by the gastric juice; secondly, the mucosa of the mouth has a remarkably good blood supply, and it is a general rule that the better the blood supply of a tissue, the less liable it is to invasion by pyogenic bacteria; thirdly, as Bloomfield (1922*c*) has shown, there is a highly efficient mechanism, apparently dependent on suction currents, by which any bacteria introduced into the mouth are drawn directly backwards towards the œsophagus, avoiding the tonsils and posterior wall of the pharynx. Of the great number of different organisms found in the mouth and saliva, among the commonest are (1) Micrococci, some of which form pigment and some of which do not; the non-pigment-forming micrococci include some that are anaerobic (Ozaki 1915); (2) Staphylococci, especially a white pigmented type; (3) Streptococci, either of the α-hæmolytic type, such as *Str. viridans*, or of the indifferent type; (4) Gram-positive bacilli, often arranged in chains, and belonging to the group of aciduric bacilli, or of aerobic spore-bearing bacilli; (5) Gram-negative bacilli, including organisms of the coliform and *Proteus* groups; (6) Spirochætes; these are almost invariably present between the gums and the teeth; several types have been described, such as *Treponema buccalis*, *Treponema dentium*, *Treponema intermedium* (Dobell 1912), *Treponema microdentium*, *Treponema macrodentium* (Noguchi 1912), Vincent's spirillum, and Miller's

spirillum. Organisms of the *Actinomyces* and *Fusiformis* groups are not uncommon (see Brooke 1938, Rosebury, Epps and Clark 1944, and Chapter 18).

The *intestinal flora* of the breast-fed *infant* is relatively simple, consisting largely of *Lactobacillus bifidus*; this bacillus in the early weeks of life may constitute 99 per cent. of the total organisms in the fæces (Cruickshank 1925). There are in addition a few enterococci and Gram-negative coliform bacilli (Tissier and Dreyfus 1925). Snyder (1940), who followed the changes in fæcal flora of infants from birth until some weeks after weaning, confirmed the predominance of *L. bifidus* in breast-fed infants, but found it also in 25 per cent. of the weaned infants. Coliforms and enterococci were also present in the breast-fed, but their frequency increased with weaning, and at the same time clostridia and members of the *Fusiformis* group became characteristic features of the fæcal flora. It is of interest that *Staph. aureus* was found in about 8 per cent. of the fæces at all stages—a frequency which may reflect the readiness with which the infant's nose is colonized by this organism soon after birth (see nasal flora, below).

The intestinal flora of bottle-fed infants is not so simple. *L. bifidus* is uncommon; on the other hand, another member of the aciduric group of bacteria —*Lactobacillus acidophilus*—is usually present in large numbers (Tissier 1900). Different types of coliform bacilli, enterococci, Gram-positive aerobic spore-bearing bacilli and anaerobic bacilli, occur more or less abundantly. Two moderately pathogenic clostridia, *Cl. capitovale* and *Cl. difficile*, were first discovered in the stools of normal infants (Snyder and Hall 1935, Hall and O'Toole 1935, Snyder 1937).

In the *adult*, numerous workers have found that the empty *stomach* is generally sterile. Immediately after a meal it contains numerous organisms, which have been ingested with the food; but these, with the exception of acid-resistant vegetative bacilli and sporing bacteria, appear to be killed off rapidly. If, however, the motility of the stomach is excessive, or the acidity is below normal, this sterilizing effect of the gastric juice is probably incomplete. Thus in cases of gastric disease—particularly of carcinoma—sarcinæ, saprophytic bacilli, and other organisms may actually multiply in the stomach (Goodsir 1842, Oppler 1895). In the healthy adult there appear to be very few organisms in the duodenum and upper jejunum. Below this level they increase, and reach their maximum numbers in the large intestine.

The organisms present in the upper part of the *small intestine* appear to consist chiefly of enterococci. In the lower part numerous other organisms are found, and in much larger numbers; amongst these are Gram-negative coliform bacilli, staphylococci, various gelatin-liquefying organisms, sarcinæ, yeasts, and sometimes aciduric bacilli (Niszle 1928). In the *large intestine* the flora is even more complex and numerous. The following organisms are commonly found: coliform bacilli of different types, enterococci, staphylococci of both the *aureus* and *albus* varieties, anaerobic spore-bearing organisms such as *Cl. welchii* and *Cl. putrificum*, aciduric bacteria including *L. acidophilus* and *L. brevis*, thermophilic bacteria, spirochætes (Werner 1909), and yeasts; less frequent are *Proteus* bacilli, *Ps. pyocyanea*, organisms of the Friedländer group, and aerobic spore-bearers such as *B. subtilis* and *B. mesentericus*. Hæmolytic streptococci of Groups D, B, C, F and G are occasionally found, Group D being the commonest (Smith and Sherman 1938). The proportion of anaerobic to aerobic bacilli in the intestine has probably been underestimated by most workers. Anaerobic organisms appear, in fact, to be very

common in the fæces. Eggerth and Gagnon (1933) and Eggerth (1935), examining 65 specimens of normal stools, found that in nearly all of them the predominating organisms were obligate non-sporing anaerobes. The exact classification of these organisms was open to doubt; some were Gram-positive and some Gram-negative. It is probable that they belonged partly to the *Fusiformis* and partly to the *Lactobacillus* groups. These findings have been confirmed by Weiss and Rettger (1937), and Misra (1938). It may be noted that bacteria are said to make up 1/4–1/3 by weight of the fæces; the greater part of them seem to be dead.

It is of interest to consider why the upper part of the small intestine contains so few living bacteria. It appears that the "*auto-disinfecting mechanism*" of the duodenum—as described by Arnold and Brody (1926)—is dependent on the acid derived from the stomach. In normal persons the H-ion concentration of the duodenum and upper jejunum is about pH 5·5–6·3. The gastric contents entering the duodenum are saturated to their full buffer capacity, usually with an excess of free acid. The free acid is quickly neutralized by the bile, and by the pancreatic and intestinal juices; the acid-buffered material is neutralized more slowly, and is chiefly responsible for maintaining the acidity of the contents of the upper part of the small intestine. In the presence of this degree of acidity few organisms are able to multiply; but if, from gastric hypoacidity, the reaction of the duodenum becomes neutral or slightly alkaline, a rich bacterial flora develops. This can be imitated experimentally in dogs by injecting alkaline buffered solutions into the duodenum, by feeding with alkaline salts, or by raising the external temperature (Arnold and Brody 1926).

Dack and Woolpert (1932), obtained rather different results with monkeys. They found that *Chromo. prodigiosum* introduced by fistulæ into different parts of the intestine disappeared just as rapidly as when given by the mouth and made to pass through the stomach. Estimations of pH at different levels showed that the stomach contents had a pH approximately of 4–5, the duodenal of 6–7, the jejunal of 6–7·5, the ileal of 6–7, the cæcal of 5–7, and the descending colon of 5·5–7. In experiments *in vitro* a pH of about 4 was required to bring about a rapid reduction in the number of these organisms. This pH was met with only in the stomach. If, therefore, bacterial destruction occurs as rapidly in the intestine of the monkey as in the stomach, it is clear that acidity cannot be solely responsible. Bergeim has described two antibacterial mechanisms in the cæcal contents of rats and man, one dependent on volatile fatty acids—formic, acetic and butyric—the other on H_2S. At the pH of the cæcal contents, butyrate from a high butter fat diet was found to act slightly on *L. acidophilus* or yeasts, and more strongly on *Bact. coli*, *Proteus vulgaris*, and salmonellæ. If rats on this diet were given $CaCO_3$ to raise the pH of the intestinal contents, the proportion of *Bact. coli* in the fæces increased. In concentrations that occurred naturally in the cæcum of rats and man, H_2S killed yeasts, and inhibited *Bact. coli* to some extent, but hardly affected *L. acidophilus* or the enterococcus (Bergeim 1940, Bergeim *et al.* 1941).

Provisionally we may conclude that in healthy persons the majority of the organisms ingested in the food and swallowed with the saliva are destroyed in the stomach by the gastric juice. The few surviving bacteria that pass through unharmed find the conditions in the duodenum and upper jejunum too acid to allow them to multiply. With their further passage down the intestine, they encounter progressively more alkaline conditions, and are able to proliferate accordingly (see Chapter 45).

The variations of intestinal flora within mammalian species may be very great. For example, the normal flora of the guinea-pig intestine was found by Crecelius and Rettger

(1943) to be relatively simple; a lactobacillus resembling *L. acidophilus* constituted approximately 80 per cent. of the total cultivable flora, the remainder being accounted for mainly by soil and air bacteria, yeasts, and an anaerobic sarcina. Coliform organisms and enterococci were seldom present. Again, according to Porter and Rettger (1940) the flora of the white rat on a normal mixed diet consists mainly of lactobacilli and coliform organisms; very few cocci are found. Lactobacilli were common in the stomach, where little else but yeasts were demonstrable. The bacterial content of the duodenum and jejunum was low, and no lactobacilli were present. Coliforms and lactobacilli were present in the lower parts of the small intestine, and abundant in the large intestine, where spore-bearing anaerobes also appeared. The lactobacilli were generally reduced by meat, to some extent by other types of high protein diet, and by several days' starvation. Except in the lower gut, it may be noted, there was little correlation between the pH of the gut contents and the predominating flora.

Origin of the Alimentary Flora.—Witkowski (1935), investigating the origin of the mouth flora, found that organisms could be demonstrated in the mouths of 49 out of 50 infants shortly after birth. These comprised mainly *Staph. albus*, coliform bacilli, Döderlein's bacillus, and streptococci. Examination of vaginal swabs from the mothers showed that these organisms were all present in the vagina in proportions corresponding fairly closely to those in the infant's mouth. It seems probable, therefore, that the initial flora of the infant's mouth is derived from the vagina of the mother. By 2–5 days after birth other organisms had appeared consisting, in decreasing order of frequency, of pneumococci, hæmolytic staphylococci, various types of streptococci, Friedländer bacilli, corynebacteria, sarcinæ, and Gram-negative cocci. Comparison of these findings with the mouth flora of the mother showed a considerable degree of correspondence, suggesting that the infant had derived these additional organisms from the nasopharyngeal tract of its mother and other persons in its environment.

The intestine at birth is either sterile, or contains a few bacteria; infection rapidly occurs, partly from above and partly from below.

Schild (1895) found that the anus became contaminated with organisms from the air, bath-water, and other objects with which it came into contact. Organisms could be demonstrated in the meconium sometimes within 4 hours of birth, and usually within 10–17 hours. When the anus was disinfected and covered with a sterile towel, the meconium remained free from organisms for 20 hours—by which time infection had occurred from the food.

The sterility of the meconium observed by Schild was confirmed by several observers (see, for example, Passini 1932), though a number of others have cultivated bacteria from 10–38 per cent. of samples examined soon after birth, indicating the possibility of small degrees of prenatal contamination of the fœtus (Hymanson and Hertz 1917, Burrage 1927, Hall and O'Toole 1934, Snyder 1936).

The Importance of Micro-organisms in the Intestine.

It was at one time widely believed that the presence of bacteria in the intestine was essential for the life of the host, that they assisted in the digestive processes, and that without them much of the food taken in would be passed out of the body again in an unassimilable condition. This belief arose as a very natural deduction from Pasteur's work on the microbial nature of fermentation. It was largely owing to Metchnikoff, who came to regard the intestinal flora with hostile suspicion, that the possibility of maintaining life with a sterile intestinal tract began to be considered seriously. Numerous workers had already devoted themselves

to the realization of this possibility; notable amongst them was Schottelius (1899, 1902, 1908), who spent several years endeavouring to rear chicks under sterile conditions, and failed. Nuttall and Thierfelder (1895–96) met with some success; they removed an embryo guinea-pig by Cæsarean section, and maintained it uncontaminated for 8 days, after which the experiment had to be given up owing to technical difficulties. They convinced themselves, however, that bacteria in the intestinal canal are not necessary for life—so long, at least, as an animal diet is provided. It was Cohendy (1912) who finally succeeded in showing that prolonged life was possible under such conditions. By means of a specially constructed apparatus, he reared chicks under completely sterile conditions for from 12 to 40 days, and found that they developed and grew as well as control non-sterile chicks kept in a similar apparatus. The only difference noted was that the food of the sterile chicks was less perfectly digested than that of the control chicks; but the sterile chicks made up for this by eating more. Cohendy concludes that life is possible with a sterile intestinal tract, but that the intestinal bacteria are probably of use in assisting digestion. How far the second part of this conclusion is applicable to man, whose diet contains very much less indigestible material than that of the chick, it is impossible to say. Cohendy states that his sterile chicks survived exposure to moisture, hunger, and thirst for several days; whereas normal chicks proved extremely susceptible to such conditions. His explanation is that, as the chicks were free from all micro-organisms, they could not, however low their resistance might be, succumb to infection from the intestine or other parts of the body.

It is easy to pass from the conclusion that bacteria are unnecessary for the human organism to the conclusion that they are actively harmful. This is a transition that has been unjustifiably made. We have no space to discuss the question of *intestinal toxæmia*. The conception that numerous cases of ill-health and of actual well-defined disease processes are due to the absorption, from the intestine, of toxic products elaborated by proteolytic micro-organisms is a plausible one, but one that rests largely on unproven assumptions. It is the almost complete lack of experimental evidence that renders the discussion of this subject so fruitless at the present time (see Discussion 1913, Dudgeon 1926).

The earliest method attempted in efforts to prevent the possible absorption of toxins from the intestine, was the replacement of the putrefactive flora—anaerobic spore-bearers such as *Cl. sporogenes*, *Cl. putrificum*, and *Cl. histolyticum*; aerobic spore-bearers such as *B. mesentericus*; and organisms such as *Proteus vulgaris* and *Ps. pyocyanea*—by an organism of the aciduric group known as *Lactobacillus bulgaricus*. These attempts failed completely. It has since been found impossible to implant this bacillus in the normal intestine; the conditions for its growth are unfavourable. Recent experimental work has shown that, in order to encourage the growth of a given type of organism in the intestine, it does not suffice to administer such an organism by the mouth, but that it is also essential to give by the mouth a sufficient quantity of a selective food material to enable it to flourish at the expense of other organisms. The replacement of a proteolytic by a saccharolytic flora—aciduric bacilli, *Cl. welchii*, and the enterococcus—can be largely accomplished by feeding on a high carbohydrate diet. In rats it has been found that by giving large quantities of lactose or dextrin in the diet, the intestinal flora can be so changed that it contains 90 per cent. or more of aciduric bacilli (Rettger and Cheplin 1921, Cannon and McNease

1923, Cruickshank 1928). These two carbohydrates are absorbed very slowly, and consequently pass into the large intestine. They are there acted upon by the aciduric bacilli—notably *L. acidophilus*—with the formation of a large amount of lactic acid ; the presence of this acid is unfavourable to the persistence of the proteolytic bacteria. Without the supply of lactose, dextrin or milk the aciduric bacilli cannot be successfully implanted in the intestine, even though pure cultures are given in large quantity daily. In infants 12 per cent. of lactose must be added to whole cow's milk or to lactic acid milk in order to convert the mixed intestinal flora into a lactobacillary flora similar to that found in the stools of breast-fed babies (Gerstley, Howell, and Nagel 1932). This is not the place to discuss the value of changing the intestinal flora in the treatment of constipation and other disorders, but we will refer our readers to the monograph by Rettger and his colleagues (1935). (See also Sanborn 1931*a*, *b*, Kopeloff *et al.* 1932, Weinstein *et al.* 1938.)

In the normal human adult, living on a mixed dietary, no sharp division into proteolytic and saccharolytic bacteria in the fæces can be made. As Dudgeon (1926) points out, the term " proteolytic " is often used without any definite understanding of its connotation. Many workers include *Bact. coli* in the proteolytic flora, which is quite inadmissible ; *Proteus vulgaris*, and *Ps. pyocyanea*, both of which are proteolytic, are uncommon in the fæces ; anaerobic bacteria, such as *Cl. sporogenes* and *Cl. putrificum*, are demonstrable in only a small proportion of samples ; while the clostridium that is most commonly present, *Cl. welchii*, has only weakly proteolytic powers. Dudgeon lays emphasis on the importance of diet in regulating the intestinal flora (see also Torrey 1930) ; he would regard, in fact, the normal flora as being determined largely by the number of organisms ingested, and by the favouring or inhibitory effect of various articles in the dietary on the multiplication of one or more types of bacterium already present in the intestine. In the practical examination of the intestinal flora, he considers it essential for the patient's diet to be rigidly controlled, and for several methods of investigation—microscopical observation of films stained by different methods, and aerobic and anaerobic cultivation in different media—to be employed.

Intestinal Bacteria as Food-stuffs. Although the limited experiments with animals reared in a sterile environment indicate that bacteria are not necessary for the human organism, there is growing evidence that in certain circumstances the intestinal bacteria may contribute to the well-being of the host.

Thus, on diets deficient in members of the Vitamin B complex, an increase in these vitamins, in some cases up to 70-fold, has been demonstrated in the rumen of cows and ewes (Bechdel *et al.* 1928, McElroy and Goss 1939, Wegner *et al.* 1941). Bechdel found a flavobacterium, constituting 90 per cent. of the flora of the rumen, which proved to be a potent source of the B complex when grown in a vitamin-free medium. Again, in a number of heifers fed with urea and carbohydrate, protein was synthesized, apparently by various iodophilic bacteria, in amounts sufficient to satisfy a substantial part of the protein requirements of the animal (Pearson and Smith 1943, Smith and Baker 1944).

The synthesis of vitamins in substantial amounts is not a peculiarity of bacteria in the rumen of herbivores. *In vitro*, the intestinal bacteria of man—for instance *Bact. coli* and *Bact. aerogenes*—synthesize biotin, riboflavin, thiamin, nicotinic acid (Burkholder and McVeigh 1942) and Vitamin K (Dam *et al.* 1941). Synthesis *in vivo* has been demonstrated in two ways, namely by noting the continued excretion of a vitamin in an animal upon a vitamin-free diet—depletion of the body reserves being excluded as a possible source ; and by the induction of avitaminosis by partial sterilization of the intestinal contents with sulphaguanidine or succinyl sulphathiazole, followed by the cure of the avitaminosis during drug-therapy by giving adequate doses of the appropriate vitamin.

The sulphonamide is said to act by reducing the number of vitamin-synthesizing bacteria, or (Gant *et al.* 1943) by reducing their ability for synthesis.

The results so far obtained by these methods strongly suggest that in the alimentary canal of the rat, Vitamin K (Black *et al.* 1942), biotin, folic acid (Nielsen and Elvehjem 1942), the B-complex, Vitamin E (Daft and Sebrell 1942), and possibly certain essential amino-acids (Martin 1944) are synthesized by bacteria and used by the host. In man, a similar dependence upon intestinally-synthesized thiamin (Najjar and Holt 1943; but see Alexander and Landwehr 1945), riboflavin (Najjar *et al.* 1944) and nicotinic acid (Ellinger, Coulson and Benesch 1944) has been demonstrated. It is not yet known whether the intestinal organisms need minimal quantities of the vitamins in the diet to initiate the synthesis. Nor is it known whether the vitamins so manufactured are necessarily absorbed as such; but we may note the suggestion of Petri, Norgaard and Bandier (1940) that the synthesis of co-enzymes I and II from nicotinic acid, of cocarboxylase from thiamin and of riboflavin from flavoproteins, takes place in the stomach.

As we have seen in Chapter 54, the fact that a substantial amount of the vitamin or food requirements of an animal may be satisfied by the bacteria in its alimentary canal affects the interpretation of a good deal of recorded work on experimental variations of diet. It also throws light on the association of deficiency states with diarrhœal syndromes, and with prolonged therapy of intestinal infections with drugs like sulphaguanidine and succinyl sulphathiazole.

Intestinal bacteria may also destroy essential food-stuffs. Thus, in the absence of a more readily fermented carbohydrate, *Bact. coli* and a number of other intestinal species will decompose Vitamin C in media containing organic nitrogen. It is not known to what extent dietary Vitamin C is destroyed in the alimentary canal, but the hypothesis of its decomposition by bacteria is in accord with the observation that certain scorbutic patients respond to injected, but not to oral, Vitamin C (Young 1942, Young and James 1942, Young and Rettger 1943). Again, nicotinic acid may be utilized as the sole source of nitrogen by organisms of the *Pseudomonas* group and by certain chromobacteria, though in the absence of nicotinic acid, these organisms synthesize the vitamin (Koser and Baird 1944; see also Benesch 1945). The production of a vitamin by the intestinal flora will clearly be determined by a multiplicity of factors, of which the presence of vitamin-producing and vitamin-destroying bacteria is only one; and the determination of these factors presents an exceedingly complex problem in bacterial ecology.

The Normal Flora of the Respiratory Tract.

For our present purpose we may divide the respiratory tract into three sections: an upper part, including the anterior and posterior nares and the nasopharynx; an intermediate zone, common to the respiratory and alimentary tracts, including the oropharynx and the tonsils; and a lower part, including the larynx, trachea, bronchi and lungs.

The bacterial flora of the nasal passages differs in several respects from that of the nasopharynx. It is less copious: if swabs are taken from the nose and nasopharynx of a sample of normal persons, and plated on some suitable medium, the nasal swabs will give the lower colony counts, and the differences observed are often striking. Qualitatively, diphtheroid bacilli and staphylococci—both *Staph. aureus* and *albus*—are far more frequent in the nose than in the nasopharynx, while *Str. viridans*, indifferent streptococci, and Gram-negative cocci of the *N. pharyngis* type, are far less frequent. These non-hæmolytic streptococci and Gram-negative cocci appear to constitute the basal normal flora of the naso-

pharynx in most communities, so far as this is revealed by the usual methods of cultivation; and it would seem that the flora of the oropharynx is composed mainly of the same species (Neumann 1902, Shibley *et al.* 1926, Noble *et al.* 1928, personal observations).

It would appear (Calamida and Bertarelli 1902) that the accessory nasal sinuses are normally sterile. According to Thompson and Hewlett (1896), the trachea and bronchi contain few, if any, bacteria. It is, of course, clear that a certain number of bacteria must be drawn through the larynx with the inspired air; but it would seem that the highly efficient filter provided by the tortuous nasal passages removes the great majority of the bacteria entering the nose. Those which penetrate beyond the vocal cords are promptly removed; in part perhaps by being swept upwards again by the cilia of the lining epithelium; in part by being caught up in the mucous secretion, and subsequently removed by expectoration; in part by removal, *via* the lymphatics, to the regional lymphatic glands. In any case it would appear that, in healthy persons, and under normal conditions, there is no permanent bacterial colonization of the tracheal or bronchial mucosa (see Nenninger 1901, Paul 1902, Quesnil 1902).

The diphtheroid bacilli and staphylococci which are usually present in cultures from the nose, and the non-hæmolytic streptococci and Gram-negative cocci of the nasopharynx and tonsils, are seldom the only species isolated from swabs taken from these situations. Various other species, including many potential pathogens, are often found. The relative frequency of three such species—*Str. pneumoniæ*, hæmolytic streptococci and *H. influenzæ*—in the nose, nasopharynx, tonsils, and oral cavity in general, is shown in Table 192.

TABLE 192

SHOWING THE FREQUENCY OF CERTAIN BACTERIAL SPECIES IN THE NASOPHARYNX, NOSE, TONSILS AND ORAL CAVITY AMONG A SAMPLE OF THE GENERAL POPULATION (Report 1930.)

Group.	No. of Swabs.		Pneumococcus.	*H. influenzæ*.	Hæmolytic Streptococci.	Gram-negative Cocci.
			Per cent.	Per cent.	Per cent.	Per cent.
I and II .	412	Nasopharynx	40·98	59·01	7·77	97·09
		Nose	16·79	11·68	0·05	12·11
III . .	137	Nasopharynx	42·34	59·54	5·84	97·08
		Tonsil	44·53	54·89	5·11	98·54
IV . .	252	Nasopharynx	49·80	51·01	11·90	95·24
		Mouth-wash	25·70	25·10	11·95	94·82

These figures were obtained during an investigation carried out in Manchester between 1925 and 1927, and refer to a series of observations in which swabs were taken from the nasopharynx of a large number of persons over a total period of 2 years. In a proportion of these persons a second swab was taken at the same time, from the nose in two groups, and from the tonsils in a third group; in a fourth group a general mouth-washing was collected and examined, at the same time as the nasopharyngeal swab was taken. Cultures were prepared on blood agar and Fildes' agar, and mice were inoculated intraperitoneally with each specimen. Figures showing the frequency of Gram-negative cocci are included for comparison. It will be noted that all these species were far less frequent in the nose than in the nasopharynx. This difference is particularly striking in the case of the normal Gram-negative cocci; but it is clear that there is far less

tendency for the pathogenic species to vegetate in the nose than in the nasopharynx. Mouth-washing, in this series of cases at least, appears to be a less effective method of isolating the pneumococcus or the influenza bacillus, but equally effective as regards the hæmolytic streptococci, or the Gram-negative cocci (see also Gundel and Linden 1931, Gundel and Okura 1933, Gundel 1933).

It must not be supposed that these are constant figures. They fluctuate rather widely from time to time and from place to place. Thus the nasopharyngeal carrier rate of hæmolytic streptococci may vary from zero to 20 per cent. in a sample of the normal adult population subjected to repeated swabbing. The fluctuations in pneumococci and influenza bacilli are usually smaller.

Table 193 summarizes the results of a large survey by Straker, Hill and Lovell (1939), which included a group of adults sampled at intervals over a period of seven years. In children, the pneumococcal carrier rates tended to be higher than those recorded in the table, but otherwise the rates were not significantly different.

TABLE 193

SHOWING THE CARRIER RATES FOR CERTAIN BACTERIAL SPECIES IN THE NASOPHARYNX AND NOSE FOR A GROUP OF ADULTS IN AN URBAN POPULATION

Organism.	Nasopharyngeal Carrier Rate.	Nasal Carrier Rate.
	Per cent.	Per cent.
Str. pneumoniæ	20–40	5–15
Hæmolytic streptococci	5–15	< 1
H. influenzæ	40–80	5–10
N. meningitidis	5–20	0–4
Gram-negative cocci other than *N. meningitidis*	90–100	0–15

The rates varied to some extent with the season. In the nasopharynx, pneumococci and to a slight extent *H. influenzæ* were found more frequently in cold, damp seasons than in the dry hot periods; in the nose a similar, but more definite seasonal variation occurred. There was some evidence of an association between these two organisms, whose colonization of the nasopharynx appears to be fairly constant; in the damper colder seasons, they appeared more frequently in the nose of living persons, and in the trachea of persons who had died from a variety of causes. That is, in the summer months the organism was confined largely to the nasopharynx, but in late winter and early spring it tended to colonize downwards into the trachea and forward into the nasal cavity.

The carrier rates for hæmolytic streptococci in the throat are substantially the same in other countries as in Great Britain (see, for example, Bryce and Tewsley 1938, Wu 1941, Boisvert 1942, and Chapter 66). An association of high carrier rates with the possession of tonsils, both in children and adults, has been noted by a number of workers. Thus, Burpee (1937) in an institutional survey records a rate of 27·3 per cent. in persons with tonsils, and 10·3 per cent. in those whose tonsils had been removed (see also Chesterman and Scandrett 1940). Of the hæmolytic streptococci isolated from the throat, a large proportion belong to Lancefield's Group A. The proportion varies from 20–70 per cent. (see, for example, Fleming 1939, Pomales-Lebrón 1940, Harper and Williams 1944), some of the remainder belonging to Lancefield's other groups. It appears that Group A streptococci colonize the tonsils more readily than other hæmolytic streptococci, for in tonsils excised as a routine, Macdonald and others (1940) found Group A streptococci over three times as frequently as those of other groups. Rantz (1941) obtained similar results, and suggested that streptococci other than Group A were to be regarded as transient inhabitants of the superficies of the tonsil, while Group A were colonizers of the tonsillar crypts.

Such studies often show a striking tendency for any given individual to maintain a characteristic nasopharyngeal flora over a considerable period of time. For instance, it is found that one person may carry one or more given types of pneumococcus in the nasopharynx over a period of months and years. Moreover, when pneumococci are isolated from a carrier after a long period, during which samples have given negative results, the newly appearing pneumococcus is of the same type as that originally isolated. In other cases, there may be replacement of one type by another (Gundel and Schwarz 1932, Straker, Hill and Lovell 1939, Smillie *et al.* 1943 ; see also Chapter 77).

In recent years, it has become clear that potentially pathogenic *Staph. aureus* must be included in the normal flora of the anterior nares. Carrier rates of 20 to 50 per cent. are recorded for healthy adults, and rates up to 80 per cent. for healthy nurses working in hospitals or institutions (see, for example, Hallman 1937, McFarlan 1938, Bartley 1941). In most cases, large numbers of *Staph. aureus* are present, and there are indications that the carrier state is relatively persistent (Miles *et al.* 1944), even in persons from whom the cocci are isolated only intermittently, and that the true carrier rate is in the region of 75 per cent.

The characteristic nasopharyngeal flora, with its basic constituents such as streptococci of the viridans type and *N. pharyngis*, together with the fluctuating population of potentially more pathogenic bacteria, is established soon after birth ; and there is no doubt that the infant derives these organisms from the adults or older children in its immediate entourage.

Gundel and Schwarz (1932) have recorded an interesting study of this problem. They examined 426 nasopharygeal swabs from 51 mothers, 645 from their new-born infants, and 66 swabs from nurses in attendance. In accord with earlier observations they found that the nasopharynx was sterile at birth ; but in the first 2 days it became colonized by the usual viridans streptococci, Gram-negative cocci of the *pharyngis* type, occasional diphtheroid bacilli and a certain number of coliform bacilli. By the 2nd or 3rd day pneumococci and influenza bacilli were often present, and their frequency was found to depend on their distribution among the adults in immediate contact with the child, and particularly on their presence or absence in the mother. By serological typing it was often possible to establish the identity of the type of pneumococcus present in the child with that present in the mother (see also Kneeland 1930).

In the same way, infants rapidly acquire *Staph. aureus* from their surroundings. Our colleagues, Dr. Cunliffe and Dr. Stokes (personal communication), have found that within a few days of birth infants in maternity homes and hospitals become heavy nasal carriers, apparently acquiring this organism from nasal carriers among the mothers or nurses ; carrier rates in young infants may be 100 per cent. In older children lower rates are recorded, but they are often higher than those in adults (see McFarlan 1938).

Various workers have described the presence of small Gram-negative, filter-passing, anaerobic bacilli, in suspensions obtained by washing out the nares, nasopharynx and oropharynx with sterile broth (Olitsky and Gates 1921*a*, *b*, Olitsky and McCartney 1923, Mills, Shibley and Dochez 1928, Garrod 1928, Burky and Freese 1931). The evidence suggests that these strains may be differentiated into several distinct types ; but they have not yet been submitted to detailed study from the systematic point of view. The so-called *Bacterium pneumosintes*, at one time regarded as a possible cause of epidemic influenza, apparently belongs to this group. The data at present available with regard to the relative frequency of these bacilli in normal persons, and in those suffering from colds, does not indicate that they play any significant rôle in such

infections. Garrod (1928) has also described the isolation, by the same technique, of minute, Gram-negative, filter-passing, anaerobic cocci

The Normal Flora of the Vagina.

An important distinction must be drawn between the flora of the vulva and vestibulum on the one hand and that of the vagina proper on the other. Except immediately after parturition and during the first few days of the puerperium, the vaginal flora is quite distinct from that of the vulval flora.

The *vulva* of the newly-born child is sterile; organisms make their appearance in about 7 to 8 hours. The normal flora of the vulva is a rich and varied one, and depends largely on the organisms present in its immediate environment. According to Wegelius (1909) it consists of: (1) Obligatory aerobes and aerophilic bacteria, of which the chief types are pseudo-diphtheria bacilli and *M. tetragenus*. (2) Coliform bacilli. (3) Facultative anaerobes, usually more or less susceptible to acid. (4) Bacilli derived from the vagina, including yeasts. (5) Obligatory anaerobes. On agar plates incubated aerobically the commonest organisms to form colonies are staphylococci, diphtheroids, enterococci, sarcinæ, and coliform bacilli. Besides these organisms, yeasts—*Oidium* and *Saccharomyces*—are very common; and the smegma bacillus is not infrequently demonstrable in smear preparations stained with Ziehl-Neelsen. Under anaerobic conditions of cultivation numerous colonies appear, consisting of organisms that have as yet been only imperfectly studied. Pathogenic bacteria are uncommon.

The normal flora of the *vagina* seems to depend largely on the glycogen content of the vaginal epithelium which, in its turn, is dependent on ovarian activity (Miura 1928, Cruickshank and Sharman 1934). The vagina of the newly born child is sterile; organisms make their appearance in 12–24 hours. At first they consist of staphylococci, enterococci, and diphtheroids, but these are often replaced in 2 or 3 days by a practically pure culture of Döderlein's bacillus (see Chapter 31). At this time glycogen is demonstrable in the vaginal epithelium, and the vaginal secretion itself is acid. The occurrence of glycogen appears to be due to the presence of œstrin derived from the maternal circulation. Soon this is excreted in the urine, glycogen is no longer demonstrable in the epithelium, Döderlein's bacillus disappears, and the vaginal secretion reverts towards alkalinity. From now on till puberty the vaginal secretion remains alkaline, and there is a varied flora of staphylococci, streptococci other than *Str. pyogenes*, coliform and diphtheroid bacilli. At puberty glycogen is again deposited in the vaginal wall, the secretion becomes acid, and Döderlein's bacillus establishes itself as the predominant organism. The flora, however, still remains mixed and Döderlein's bacillus is accompanied by many of the organisms that we have just mentioned, as well as by anaerobic streptococci, which appear to be commonly present in the female genital tract (see p. 596). At the menopause, when ovarian activity ceases, the conditions revert to those met with before puberty. Following parturition, and during the first few days of the puerperium, the vaginal flora resembles that of the vulva. At the time of parturition organisms ascend from the vulva to the uterus, where they may be demonstrated for several days (Wegelius 1909).

The vaginal secretion has a marked bactericidal action (see Küster 1929). To a large extent this may be ascribed to the presence of lactic acid, though other factors are probably concerned (Chapter 54). The lactic acid itself is derived from the glycogen, partly perhaps as the result of natural enzymes, but mainly on account of the fermentative activity of Döderlein's bacillus. (Further references:

Döderlein 1892, Segre 1929, Cruickshank and Baird 1930, Pettit and Hitchcock 1933, Cruickshank 1934, Hardy 1941.)

The Normal Flora of the Urethra.

In the anterior urethra of males occasional white staphylococci and diphtheroids may be found, and a short Gram-negative diplobacillus, not to be mistaken for the gonococcus (Kutscher 1909). The female urethra is either sterile or contains a few non-pathogenic cocci. A saprophytic acid-fast bacillus, known as *Mycobacterium smegmatis*, is quite common in the preputial secretion both of males and females; this organism may occasionally find its way into the urine, and be mistaken for the tubercle bacillus.

Normal Flora of the Skin.

Under this heading we shall exclude all the multifarious organisms that may at one time or another be found on the surface of the skin, the numbers and varieties of which depend largely on personal hygiene and environmental conditions. Price (1938) distinguishes this "transient" flora from the "resident" flora, which consists of a relatively few types of bacteria which seem to multiply freely on the skin, and particularly in the sebaceous glands. The "resident" flora, which can generally be cultivated without difficulty, contains large numbers of Gram-positive cocci. Of the numerous types that have been described much the commonest is the white pigmented variety called by Welch (1891) *Staphylococcus epidermidis albus* (see Chapter 25). Another variety, distinguished by its feebler biochemical activity, is found very frequently in scurf, and is known as Gordon's scurf staphylococcus. Occasionally yellow-pigmented staphylococci are found, and sometimes sarcinæ. The great majority of the cocci found on the skin are comparatively non-pathogenic both to human beings and to rabbits, but potentially pathogenic *Staph. aureus* is not infrequently found (Koch 1908). In moister parts, like the axillæ and inguinal folds, Gram-negative bacilli often predominate.

Staph. aureus is not uncommon on the skin of the face and hands, but less common on other parts of the body. The numbers present are usually small, but carrier rates of 5–24 per cent. have been recorded (Vierthaler 1940, Bartley 1941, Smith 1941, Martin 1942).

Skin carriage is more frequent among persons who are nasal carriers. Where typing of the *Staph. aureus* isolated has been possible, either by serological or by bacteriophage tests, similar types have been found in the nose and on the skin in the majority of cases (Gillespie *et al.* 1939, Miles *et al.* 1944). To some extent, therefore, it appears that the skin is continually infected by *Staph. aureus* from the nose. This organism is usually "transient" in Price's sense, but, as Devenish and Miles (1939) showed, some persons carry *Staph. aureus* so deeply in the recesses of the skin that prolonged sweating is required to bring it to the surface. In these cases it appears to be a "resident" (see also Gillespie *et al.* 1939, Vierthaler 1940).

In Chapter 66 we have discussed *Str. pyogenes* as an occasional transient member of the skin flora. There is no reason to believe that it ever forms part of the resident flora of the clinically normal skin; and indeed, in the light of Burtenshaw's (p. 1020) demonstration of natural fatty antistreptococcal substances in the normal skin, it seems unlikely that it could ever do so.

Amongst the other organisms met with are occasional coliform and *Proteus* bacilli, and diphtheroids. The healthy skin appears to have some natural self-

disinfecting mechanism which is responsible for the rapid disappearance of living organisms implanted upon it (see Chapter 45 and Colebrook and Maxted 1933, Arnold and Bart 1934).

In the skin lining the external auditory meatus, as well as the usual staphylococci, diphtheroid bacilli are not uncommon, and sometimes saprophytic acid-fast bacilli, derived from the cerumen, are met with. In the conjunctival sac, organisms are comparatively scanty, and consist chiefly of diphtheroids, such as *C. xerosis*. The conjunctiva may owe its relative freedom from bacteria, in part, to a highly potent lytic enzyme, called by Fleming and Allison (1922) *lysozyme*. These workers found that tears have a high bactericidal power, capable of dissolving certain saprophytic cocci in a dilution of 1/40,000. Further study showed that, with the exception of cerebrospinal fluid, sweat, and urine, this enzyme is distributed to a greater or less extent in practically all the human secretions and tissues, as well as in animal and some vegetable tissues.

Testing the bactericidal power against a large non-pathogenic coccus, called by them *M. lysodeikticus*, they found the blood serum active in a dilution of 1/270, nasal mucus and sputum in 1/13,500, and saliva in 1/300. Extracts of tissues made by grinding up 1 gm. of tissue with 20 ml. of saline showed that the liver and the tonsil were active at a dilution of 1/400, the kidney and skin at about 1/100, while cartilage was active in 1/1,300. Lysozyme dissolves both dead and living organisms, and is more active as a rule against non-pathogenic than against pathogenic bacteria. Its range of activity appears, however, to be very limited, as judged by the small number of bacterial species which it attacks (see Fleming and Allison 1927, Fleming 1929, and Chapter 45).

Bacteria in the Blood and Internal Organs.

We have unfortunately very little information as to the occurrence of bacteria in the blood and viscera in healthy persons. The technical limitations of the usual method of performing blood cultures are such that small numbers of organisms may quite easily escape detection. Even if a sufficiently large sample of blood is withdrawn, it is difficult to ensure that the few organisms contained in it will grow; they may be destroyed by the bactericidal power of the blood or ingested by leucocytes. Moreover the blood that is examined is usually drawn from a peripheral vein, coming from one limited portion of the body, and not therefore so likely to contain bacteria as blood from, let us say, the pulmonary artery through which the blood from all parts of the body has to pass. It has been stated that bacteria frequently gain access to the blood stream under normal conditions, but are filtered off in such organs as the liver, lungs, and spleen, where they are destroyed by cellular and bactericidal action. We do not know how frequently this occurs, what the channels of transit are, or what bacterial species may be concerned. It is also stated that in the new-born infant the epithelial lining of the intestinal mucosa is deficient in places, and that passage of bacteria into the lymph stream and blood is very much more frequent during the first few days of life than it is subsequently. Whether the normal intestinal mucosa of the adult presents a constant barrier to the passage of bacteria is not definitely known; nor is it known why some organisms are able to pass through it very much more easily than others. Experiments made by Arnold (1928) on dogs suggest that the permeability of the mucosa varies from time to time, and is influenced by such factors as the H-ion concentration of the intestinal contents and the amount of protein in the food. It is probable too that variations in the blood supply are of importance. There is, however, good evidence that organisms, particularly

streptococci, pass into the blood stream of patients suffering from focal infections in different parts of the body (Chapter 68).

Examinations of normal mesenteric lymph glands have been few. It seems not improbable that bacteria of different types are frequently absorbed through the intestinal mucosa. Some of these are probably destroyed in the mesenteric glands or the liver; a few may perhaps make their way to the blood stream, from which they will be removed by phagocytic cells. In support of this possibility is the observation that the muscles and viscera of apparently healthy cattle and pigs not infrequently contain bacteria (Zwick and Weichel 1911, Reith 1926).

The now common diagnostic practice of sternal puncture also offers a means of exploring the bacterial content of the reticulo-endothelial system in healthy persons. So far, its bacteriological use has been confined to culture of marrow samples as an aid to diagnosis in infections where the blood culture is sterile. A wide field of experimental work is open to the student who is willing to study the distribution of living organisms in the tissues of apparently normal animals.

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CHAPTER 91

THE BACTERIOLOGY OF AIR

In Chapter 59 we have already discussed the apparently conflicting views of Cornet and of Flügge on the respective parts played by dust and by droplets in the spread of pulmonary tuberculosis. More recently observations in this country and in the United States have focused attention on the part played by aerial infection in the causation of other respiratory diseases. Work is now proceeding actively on aerial microbiology, and particularly on the ways in which spread of infection by dust and droplet nuclei can be controlled. We shall attempt to give a brief résumé of the present position, and refer our readers for a fuller account to the Symposium on Aerobiology held at Chicago in 1942 (Report 1942).

Outdoor Air

The degree of bacterial contamination of the outside air depends on a number of factors, such as the density of the human and animal population, the amount of vegetation, the nature of the ground or soil, the temperature and humidity of the atmosphere, the extent and direction of wind currents, and so on. Aeroplane surveys (see Proctor 1934, 1935, Proctor and Parker 1938) show that bacteria in the upper air consist largely of aerobic spore-bearing bacilli, and to a much less extent of organisms belonging to the *Achromobacterium*, *Sarcina*, and *Micrococcus* groups, suggesting that they are derived mainly from soil and surface dust. These organisms can be carried vertically for several miles into the air, and it is possible that they may also travel long distances horizontally. It is conceivable therefore, that certain resistant pathogenic bacteria like the anthrax bacillus, and viruses like the virus of foot-and-mouth disease, might be transmitted from one area or country to another, and serve, on again reaching the ground, as infective agents for man or animals. There is, however, no evidence to prove that this is more than a possibility. On the contrary, most of our observations suggest that infective material is seldom carried for more than short distances by air, or that, if it is, it is too diluted to cause recognizable disease. Though autotrophic bacteria may possibly undergo some multiplication in cloud areas, where moisture and traces of gases such as NH_3 and CO_2 are present, it seems extremely improbable that growth of pathogenic organisms is likely to occur in air. For the medical bacteriologist the importance of the flora of the outside air still remains to be demonstrated. On the other hand, the flora of the air inside buildings, where ventilation is limited, appears to play a major part in the causation of respiratory infection.

Indoor Air

Air-borne bacteria may be distributed in three different forms: (*a*) attached to dust particles; (*b*) contained in gross droplets expelled from the nose and

mouth ; (*c*) present in droplet nuclei, which result from evaporation of the smaller droplets expelled from the nose and mouth.

Dust consists of varying sized particles of animal, vegetable, or mineral origin. The heavier particles fall rapidly to the ground, but can be stirred up by sweeping and draughts ; the lighter particles, which may be 1 μ or less in diameter, remain more or less permanently suspended in the atmosphere. It has been shown by White (1936), Cruickshank (1938), Hare (1941), Thomas (1941*b*) and others that hæmolytic streptococci may be found in the dust of wards harbouring cases or carriers of these organisms. Tubercle bacilli have often been demonstrated in the dust of sanatoria (see Pressman 1937). Wright, Shone and Tucker (1941) and Crosbie and Wright (1941) have demonstrated diphtheria bacilli in large numbers in floor dust collected near diphtheria patients. Under favourable conditions these three organisms have been shown to remain alive and virulent for many weeks. Dust from bedclothes is one of their most prolific sources.

Since Flügge (1899) first drew attention to the rôle of minute droplets expelled from the mouth and nose of phthisical patients in conveying tuberculosis, the concept of droplet-borne infection has been widely applied to the transmission of respiratory disease in general. Of late years renewed interest in its mechanism has been stimulated by the studies of Wells and his colleagues in the United States (Wells 1934, Wells and Wells 1936, 1938). The observations of these workers, and of others such as Weyrauch and Rzymkowski (1938) and Jennison (1942), have amplified the earlier findings of Lange and Keschischian (1925) and Strauss (1926), and have thrown fresh light on the size and fate of droplets emitted from the mouth during sneezing, coughing, whistling, singing, and talking. A brief abstract of the earlier reports has already been given in the chapter on tuberculosis (p. 1313), but it will be well here to expand this description in the light of more recent work.

During a sneeze it is calculated that something like 20,000 droplets are expelled, mainly through the closed teeth, at an initial velocity of about 150 feet per second (Jennison 1942, Hatch 1942). Their diameter varies from about 10 μ to over 1 mm. The fate of these droplets depends on their size. The larger ones are projected some distance but fall to the ground before they can evaporate completely. The smaller ones, owing to the greater ratio of their surface to their volume, travel only a short distance and evaporate quickly (see Table 194).

TABLE 194

EVAPORATION TIME OF WATER DROPLETS IN UNSATURATED STILL AIR AT 22° C.
(Taken from Jennison 1942, modified from Wells 1934)

Diameter of Droplet in μ.	Evaporation Time in Seconds.
2,000	515·0
1,000	129·0
500	32·0
200	5·2
100	1·3
50	0·31
25	0·08
12	0·02

Droplets 1 mm. in diameter, if projected from the mouth of a man of average height, fall in a curved trajectory and reach the ground in about 15 feet. On

the other hand, droplets less than 0·1 mm. in diameter possess too little kinetic energy to enable them to travel more than a very short distance from the mouth, and are so little affected by gravitation that they evaporate before they have fallen to any appreciable extent. On evaporation, they become converted into what Wells calls "*droplet nuclei*," which behave very much like minute particles of smoke. In absolutely still air they fall at the rate of probably 1 to 3 feet per hour, but in the ordinary atmosphere they remain suspended almost indefinitely, being dependent for their transportation on air currents. It has been shown experimentally that micro-organisms attached to droplet nuclei are rapidly dispersed throughout rooms and even buildings, and are inhaled by anyone breathing air containing them (Wells and Wells 1936).

Trillat (1938) describes an interesting experiment in which he atomized one litre of a fluid suspension of *Chromobacterium prodigiosum* in a central court of the Pasteur Institute in Paris. A hundred agar plates were exposed for more than 100 metres around. Every single plate became infected, even those placed to windward of the court. Droplet nuclei, when inhaled, may be caught up by the nasopharyngeal mucosa, but, judging from the observations of Wells and Lurie (1941) on rabbits, a high proportion of them appear to reach the lungs. Available evidence, however, suggests that only particles less than 10 μ in diameter are able to pass directly into the alveoli. Since it is estimated that probaby 20–40 per cent. of droplets expelled in a sneeze are less than 50 μ in diameter and therefore evaporate to droplet nuclei within a fraction of a second, it follows that something like 4,000 to 8,000 droplet nuclei, many of them probably containing bacteria of respiratory origin, result from a single sneeze. (For photographs of droplet expulsion in a sneeze, see Weyrauch and Rzymkowski 1938, Bourdillon and Lidwell 1941, Jennison 1942.)

Droplets are also expelled, though in much smaller numbers, during coughing; as in a sneeze, the vast majority are not projected more than 2 or 3 feet. In talking, particularly in the pronunciation of sharp consonants like p, b, t, f, k and s, droplets are often expelled through the teeth; their average size is larger than of those in a sneeze, but their initial velocity is lower, and few travel more than a foot.

The part played by air-borne droplet nuclei in the spread of respiratory infection has been studied by Wells and Wells (1942), and is discussed in Chapter 59.

Measurement of Air Contamination

Several methods have been devised for measuring the bacterial contamination of the atmosphere. The results given by different methods are often widely at variance, mainly because of the differences in methods of sampling.

One of the simplest ways is to expose a series of agar plates in different parts of the room for a given time. Under ordinary conditions the number of colonies that develop is determined mainly by the larger dust particles and droplets, which fall to the ground in a more or less vertical direction. Unless the air is quite still and the plates are left for some hours, the droplet nuclei are scantily represented.

In an attempt to obtain a more representative sample of the smaller suspended particles, Wells, Phelps and Winslow (1937) devised an air centrifuge in which the bacteria from large measured volumes of air are deposited by centrifugal force on to the sides of a rotating cylinder lined with nutrient agar. Though this machine picks out practically all particles having a settling velocity in still air

greater than 30 feet per hour, it has been found experimentally that recovery of the smallest particles of a finely atomized culture, having a settling velocity of about 1·8 feet per hour, is only about one-third complete (Wells, Phelps, Robertson and Winslow 1942). (See also Wells 1943.) A simpler apparatus was devised by Hollaender and Dalla Valle (1939), in which measured volumes of air are drawn through a funnel and allowed to play on agar plates; and by Wheeler, Foley and Jones (1941), in which measured volumes of air are bubbled through broth in a flask, and the bacterial content of the broth is determined by plating. Various modifications of the Wells air centrifuge have been described by Finn and Powell (1942), who give an elementary mathematical treatment of the principle underlying its construction and use.

A simple and effective device for the collection and counting of air-borne bacteria is Bourdillon's slit sampler (Bourdillon *et al.* 1941). Air is sucked through a slit 0·25 mm. wide and impinges on to the surface of a rotating agar plate immediately beneath. The rate of suction is adjusted to 1 cubic foot of air per minute, and samples of 2–10 cu. ft. are collected, depending on the bacterial content of the atmosphere. Elliott (1941) devised an entirely different method, which has not yet been properly developed. Its essential features are first, the trapping of suspended particles by drawing the air sample through a water-vapour mist, and second, the condensation of water on any particles left in suspension by cooling the saturated air. This latter principle is derived from the Wilson cloud chamber, in which the path of electrons is made visible by depositing on them water droplets condensed from a saturated atmosphere by a sudden reduction in pressure.

For estimating the degree of nasopharyngeal pollution of the air, Gordon (1902–03) long ago, in his study of the ventilation of the House of Commons, suggested that *Str. viridans* should be used, in much the same way as *Bact. coli* is used for measuring the excretal pollution of water. This suggestion has been revived by American workers (Buchbinder *et al.* 1938, Wells, Wells and Mudd 1939), and may prove of value as soon as a reliable selective medium is available. *Str. viridans* is also useful for testing the efficacy of bactericidal agents designed to destroy potentially pathogenic micro-organisms in the air. It can be sprayed into a closed room, and the disinfection rate estimated by plate counts on air collected at measured intervals.

Measures for Controlling Air-borne Infection

As Andrewes (1940) points out, no method for the control of air-borne infection is as satisfactory as proper spacing of individuals combined with adequate ventilation. These enormously reduce the risk of infection, whether from droplets, droplet nuclei, or dust. When they cannot be achieved, other methods may be tried as substitutes. As yet the study of counter measures to air-borne infection is still in its infancy. The brief summary that we give here can do little more than indicate the directions in which research is proceeding.

Dust-borne Infection.—In hospital wards a large amount of dust is derived from blankets; bacteria-containing particles of fluff are freely liberated whenever the blankets are shaken. These particles rapidly settle on the floor, but rise again in a cloud on sweeping. Van den Ende, Lush and Edward (1940) found that if the floor was treated with spindle oil, the dust particles became sticky and mutually adherent, so that they could be swept away without being dispersed

again into the air. The effect of the oil was purely mechanical; it had no germicidal action on the organisms. Judged by the number of haemolytic streptococci cultured from the air after sweeping of the floor, oil treatment proved very effective. Thomas (1941*a*), who studied the air flora in canteens and hospital wards over a period of some months, found that the result of oil treatment of wooden floors was to reduce the number of organisms dispersed into the air on sweeping by about 80 per cent. The oil can be applied to wooden or to linoleum floors, but not to rubber, cement, or concrete. A fresh application has to be made every 6 or 8 weeks.

Observations by van den Ende and Spooner (1941), however, showed that, if bacterial contamination of the air in hospital wards was to be adequately controlled, treatment of the floor alone was insufficient. The bedclothes, particularly the blankets, which constituted the source of the dust, had to be treated too. It proved possible to do this by soaking them in 30 per cent. liquid paraffin in "white spirit," followed by removal of the excess in a hydro-extractor (see van den Ende, Edward and Lush 1941). Woollen materials retained about 2·5 per cent. of their weight of oil, and cotton materials about 6·5 per cent.; they were not perceptibly oily to the touch. In this way the heavy contamination of the air that normally accompanies bedmaking was avoided. Further work by van den Ende and Thomas (1941) showed that liquid paraffin in white spirit could be replaced by technical white oil emulsified very finely in water by means of suitable wetting agents, which had the advantage of imparting to the bedclothes potent bactericidal activity. The use of these oil-in-water emulsions resulted in a 99 per cent. reduction in the number of organisms liberated during bedmaking. In practice, Wright, Cruickshank and Gunn (1944) found that in order to control streptococcal cross-infection in measles wards it was essential to oil not only the floors, but also the bedclothes, sleeping garments, towels and similar articles. When this was carried out thoroughly, a substantial reduction in the cross-infection rate and in the middle-ear complication rate was effected (For technique of laundering garments with oil, see Harwood, Powney and Edwards, 1944).

Droplet-borne Infection.—When people are working in close contact with each other, infection conveyed by droplets may be controlled to some extent by the use of face masks. As demonstrated by actual photographs, masks made of fabric alone are not completely impermeable to the droplets expelled during sneezing; the larger droplets become enmeshed, but many of the smaller droplets pass through and are propelled for some distance into the air (Jennison 1942). On the other hand, if a layer of cellophane is sewn into a pocket between the inner and outer layers of a double-folded muslin mask, the passage of bacteria is obstructed (see Devenish and Miles 1939). It is very doubtful, however, whether any mask that can be worn with reasonable comfort can be relied on to prevent the inhalation of organisms in the form of droplet nuclei (Andrewes 1940).

Infection carried by Droplet Nuclei.—Measures to control true aerial infection are designed to destroy the micro-organisms attached to droplet nuclei, which are floating freely in the atmosphere. Hitherto, the main lines of attack have been (1) by ultra-violet irradiation, and (2) by disinfectant sprays and vapours.

1. *Ultra-violet Irradiation.*—The chief protagonists of ultra-violet irradiation have been Wells and his colleagues in the United States (Wells and Wells 1936, 1938, Wells 1940, 1942, Wells, Wells and Wilder 1942). The method may be applied in various ways. (*a*) Air may be re-circulated through ducts fitted with

sources of ultra-violet light and returned to the room. Though organisms in droplet nuclei may be destroyed by this method, many of those attached to dust particles will resist attack and enter the room still viable. It is advisable, therefore, to pass the air first through a cloth filter to remove the dust particles before exposing it to the ultra-violet rays. In this form, the method may be of value for supplying air to operating theatres, cinemas, restaurants and railway coaches. (*b*) Provided the eyes of the occupants are screened, the room may be irradiated directly. Very satisfactory results have been claimed by Hart (1941) from the use of this method in operating theatres. (*c*) Instead of exposing the whole room to ultra-violet light, irradiation may be restricted to the space above the level of the heads of the occupants whose eyes therefore require no protection. This method has been used with apparent success in the control of air-borne infection in school-rooms (Wells, Wells and Wilder 1942); but observations by Schneiter and his colleagues (1944) in a training school for boys showed that ultra-violet irradiation of the upper air of the dormitories had no appreciable affect on the incidence of air-borne disease, as judged by hospital admission records. (*d*) So-called ultra-violet light barriers may be used in hospitals to prevent the air of cubicles from becoming infected with the air of the general ward. In conjunction with air conditioning, this method is said to have given satisfactory results in the Cradle at Evanston, Ill. (Rosenstern 1942); but in an infants' hospital at Boston it failed to diminish the incidence of cross-infection (Brooks *et al.* 1942). In Lurie's (1944) hands an ultra-violet light barrier proved successful in preventing cross-infection of normal rabbits in one set of cages with tubercle bacilli from infected rabbits in adjacent cages.

2. *Disinfectant Sprays and Vapours.*—In the early days of antiseptic surgery Lister introduced a carbolic spray to sterilize the air over the wound. It is doubtful how far it was effective. Hewlett (1939), for example, found that to kill typhoid bacilli on paper slips, it was necessary to vaporize 35 gm. of phenol per 1,000 cu. ft. of air, and even then an hour's exposure had to be allowed. It is probable that, had the bacilli been atomized, a lower concentration of phenol for a shorter time would have proved effective; but since more powerful germicides than phenol are now known, the interest of the carbolic spray is mainly historical.

Though sulphurous acid and formalin vapours were used for terminal disinfection in isolation hospitals and sick-rooms, little progress in the destruction of bacteria in the air of occupied rooms appears to have been made till Douglas, Hill and Smith (1928) showed that it was possible to bring about the rapid death of *Bact. coli* in the atmosphere by means of a sodium hypochlorite solution sprayed into the air in very high dilution. In spite of this impressive result, no general interest in the possibilities of aerial disinfection was aroused till the publication of Wells's papers in 1934 and 1936 on the nature and significance of air-borne bacteria in the causation of respiratory disease. In 1938 appeared Trillat's paper on bacterial aerosols, and Masterman's paper on the purification of the air of inhabited rooms by a hypochlorite spray. Since then, intensive study has been devoted by several workers to the problem of finding a suitable chemical agent for disinfection of the atmosphere. Some of the more promising substances may be referred to here.

(*a*) Sodium hypochlorite. Masterman (1938, 1941) found that a reduction of over 99 per cent. in the bacterial content of the atmosphere could be brought about by atomizing 1 gm. of a 1 per cent. sodium hypochlorite solution in 40,000,000 ml.

of air. Evidence suggests that so far as bacteria of nasopharyngeal origin are concerned, such a high percentage reduction could hardly be expected.

Andrewes (1940) states that 5 ml. of a 1 per cent. hypochlorite solution were required to kill rapidly 95 per cent. of streptococci suspended in the air of a room of about 1,000 cu. ft. capacity; this gives a concentration of only 1 in 5·7 million. Pulvertaft (1944) made similar observations. Bourdillon, Lidwell and Lovelock (1942) found that 2·1 ml. of 1 per cent. sodium hypochlorite solution per 1,000 cu. ft., i.e. 1 in 13·5 million, killed almost all the bacteria projected into the air by sneezing in 3 to 4 minutes. Challinor (1943) observed a reduction of about 33 per cent. of the aerial bacteria, as measured by the slit sampler, after spraying a room with 1 per cent. hypochlorite solution in the proportion of 11 ml. to 1,000 cu. ft., i.e. a concentration of only 1 in 2·6 million. Edward and Lidwell (1943), using hypochlorous acid gas, found it necessary to use an initial concentration of 1 in 800,000 to destroy 90–99 per cent. of suspended influenza virus particles. Nevertheless, it is clear that sodium hypochlorite or HOCl has a powerful disinfectant action on most non-sporing pathogenic bacteria.

The effective concentration of sodium hypochlorite is influenced by a number of factors. A low relative humidity and a high content of organic matter in the air are both unfavourable. So also is the presence of phenol or cresol vapour. HOCl attacks metal, and is decomposed by organic matter, so that metal sprays should not be used, nor should the solution be allowed to come into contact with rubber or wool. Hypochlorite sprays should be avoided in rooms containing certain electrical equipment, such as telephone switchboards. Elford and van den Ende (1945) have pointed out the disadvantages of using hypochlorite for continuous disinfection of the air, and suggest the use of hypochlorous acid gas in its place. Even so, however, the corrosive action on metals when the gas is used at the optimum relative humidity of 70–90 per cent. may be a serious practical objection.

(*b*) Resorcinol in glycerol was found by Pulvertaft and Walker (1939) to be very effective, though too expensive for routine use. Hexyl resorcinol is also valuable. According to Twort and Baker (1942), it kills more slowly than resorcinol, but acts in a lower concentration and persists longer. It is therefore to be preferred when air changes are infrequent and overcrowding is not severe. Hexylresorcinol, in the form of a 10 per cent. solution in propylene glycol together with 0·05 per cent. sulphonated lorol, was found by Twort, Baker, Finn and Powell (1940) to be even more effective.

(*c*) Propylene glycol. This substance, which was used by Twort and his colleagues as a solvent, has itself excellent disinfectant properties. Robertson, Bigg, Puck and Miller (1942) brought evidence to show that 1 part of propylene glycol vapour in 2 to 4 million parts of air produced complete and almost immediate sterilization of an atmosphere into which pneumococci, streptococci, staphylococci, *H. influenzæ*, and influenza virus had been sprayed. Favourable results in preventing cross-infection in children's wards have been reported for propylene glycol vapour by Harris and Stokes (1942). Puck, Robertson, and Lemon (1943), who studied propylene glycol and triethylene glycol, found that both of these compounds acted best at a room temperature below 80° F. and at a relative humidity of 45–70 per cent. At low humidities the bacteria-containing droplets dried up so quickly that the glycol vapour did not condense readily on them. At high humidities water vapour condensed as well as the glycol vapour and thus diluted it. It is clear from these observations that the glycol vapours acted best on sprayed bacteria-containing droplets. Organisms in dust proved resistant.

Essentially the same conclusions were reached by DeOme and his colleagues (1944), who found that the germicidal power of triethylene glycol vapour decreased as the temperature rose from 28° to 37° C. and as the relative humidity deviated from 45 per cent. They noted also that, to produce a given reduction in the numbers of *Salm. pullorum* sprayed into the air, a concentration of propylene glycol 100 times that of triethylene glycol was required.

(*d*) Balsamic smokes. The germicidal action of the smoke given off by smouldering cardboard, tobacco, and incense was noted by Twort and Baker (1940). Incense was found to be the most effective; 1 part in 160 million of air destroyed salivary organisms within 15 minutes.

The mode of action of the vaporized disinfectants is subject to controversy. According to Trillat (1938), Pulvertaft and Walker (1939), and Twort and his colleagues (1940), atomized disinfectants exert their action in the form of minute droplets—hence the term aerosol coined by Trillat—which come into contact with the suspended bacteria. Other workers maintain, on the contrary, that they act as simple gaseous disinfectants. Masterman (1941), for example, points out that minute droplets of the size postulated by Twort, namely 0·4–1·0 μ in diameter, would evaporate in a fraction of a second (see p. 2003), and would therefore no longer be in the aerosol or mist form. So far as sodium hypochlorite is concerned, Masterman is of the opinion that its disinfectant action is due to gaseous hypochlorous acid set free by the carbon dioxide in the air. Similarly, there is growing evidence that propylene glycol acts essentially in the gaseous form. Vaporization of this substance by heat is just as effective as spraying it in the form of a mist (see Robertson, Bigg, Puck, Miller and Baker 1942). Moreover, calculation shows that the maximum number of contacts possible between the disinfectant and the bacterial droplets is insufficient to account for the extreme rapidity of sterilization so long as the disinfectant is present in the form of an aerosol. If, however, the aerosol droplets are assumed to evaporate almost at once, then sufficient molecules of gas will be liberated within 2 or 3 seconds to produce a lethal concentration of propylene glycol in the bacteria-containing droplets. This explanation is also in accord with the observation that, in the form of a liquid, propylene glycol has a very low bactericidal action, whereas in vaporized form it is among the most active disinfectants known. The term aerosol is somewhat misleading, and should probably be reserved for minute droplets of oily substances that do not evaporate in the atmosphere.

It may be mentioned that both to ultra-violet light and to disinfectant vapours Gram-negative bacilli are more susceptible than Gram-positive cocci; and that organisms of whatever nature are harder to kill when dried on to particles of dust than when present in the form of droplet nuclei.

The Control of Respiratory Infection

It is as yet too early to assess the value of the different methods just discussed for the control of respiratory disease. One of the great difficulties is to ensure the continuous absence of pathogenic organisms from the atmosphere during the 24 hours of each day. In the prevention of enteric infections reliable measures are available for the destruction of the causative organisms in water and in milk; and provided due sanitary care is exercised, food should seldom be contaminated except by accident. In this way the entire population of a city can be more or less efficiently protected. But with respiratory disease, the problem is far more

difficult. Even if good ventilation is provided in the factory, measures are taken to allay dust, overcrowding is avoided, and pathogenic organisms in the air are destroyed by ultra-violet irradiation or by disinfectant gases, the operatives are still exposed to the risk of infection in the buses, trains, and tubes that take them to and from their work, in the shops where they buy their food and other necessaries, in the places of amusement that they frequent, and probably for 10 or 12 hours of every day in the houses where they live and to which their children return bringing infection with them from school. Whether it will ever prove practicable to afford continuous protection to the worker in all these varied situations, it is impossible to say. To do so will require an intensive and prolonged education of the general public, greatly improved ventilation of houses and other buildings, and a willingness on the part of both private persons and of public authorities to incur the necessary expenditure to combat the spread of aerial infection.

For the present it will probably be wise to confine our attention to the prophylaxis of air-borne infection in such situations as operating theatres, hospital wards, out-patient departments, and certain institutions, where reliable bacteriological and epidemiological data on the type and incidence of respiratory disease can be collected. By this means we should find out in course of time how effective different measures are, alone or in combination, and be in a better position to decide whether their extension to other fields is desirable.

The prophylaxis of respiratory disease is a formidable problem, which has in the past seemed almost insuperable. We may find, however, that the complete destruction of all pathogenic bacteria in the air is not necessary, at any rate in the prevention of some diseases. It may suffice to reduce their numbers to below a critical level at which, provided they are not inhaled by the individual too rapidly or too frequently, the normal defences of the body can be trusted to deal with them. It is unlikely that all respiratory disease will be avoided. Measles and influenza will probably defeat our best endeavours for many years to come. But the abolition, at least in epidemic form, of scarlet fever and of all the varied streptococcal infections associated with it, of cerebrospinal meningitis, of whooping cough, of primary pneumococcal pneumonia, and even of pulmonary tuberculosis, though at present only a vision, may, with the application of our knowledge and in a time that is not too far distant, come to be realized as an achievement within our power.

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CHAPTER 92

THE BACTERIOLOGY OF WATER, SHELL FISH, AND SEWAGE

WATER

Bacterial Flora in Water.

It is convenient to divide the bacteria found in water into three groups:

A. Natural Water Bacteria.

In this group are included those organisms that are commonly found in waters free from gross pollution. They may be subdivided as follows:

Bacilli.

(1) Fluorescent
- Gelatin liquefied. *Ps. fluorescens liquefaciens.*
- Gelatin not liquefied. *Ps. fluorescens non-liquefaciens.*

(2) Chromogenic
- Red pigment. *Chr. prodigiosum, Chr. indicum.*
- Orange „ *Chr. aurescens.*
- Yellow „ *Chr. ochraceum.*
- Violet „ *Chr. violaceum.*

(3) Non-chromogenic: These organisms belong mainly to the *Achromobacterium* group. They are sometimes divided up according to their reactions on gelatin and milk; but they have so far not received sufficient study to render their classification possible. Gram-positive, spore-bearing aerobes, which produce acid and gas in lactose, appear to be not uncommon (Greer 1928, Porter *et al.* 1935). Members of the coli-typhoid group are not natural inhabitants of entirely unpolluted waters.

Cocci.

(1) Chromogenic. Generally yellow pigment formed.

(2) Non-chromogenic. *M. candicans, M. aquatilis, M. coronatus.*

Sarcinæ.

Chiefly *Sarcina lutea.*

B. Soil Bacteria.

These organisms, though not normally inhabitants of water, are frequently washed into it during heavy rains. Most of them belong to the group of aerobic spore-bearing bacilli, such as *B. subtilis*, *B. vulgatus*, *B. megatherium*, and *B. mycoides*. Others, such as *Bact. aerogenes* and *Bact. cloacæ*, which appear to live most frequently on grain and plants, and which may conveniently be treated as soil organisms, are aerobic non-sporing bacilli. By the use of special media other organisms, such as the nitrifying bacteria, may be isolated.

C. Sewage Bacteria.

Many of the organisms in this group are normal inhabitants of the intestine of man and animals. Others live chiefly on decomposing organic matter of either animal or vegetable origin. Occasionally pathogenic organisms are included.

(1) *Intestinal Bacteria.*
Bact. coli group.
Streptococcus faecalis.
Cl. welchii.
Pathogenic organisms, such as *Salm. typhi* and *V. cholerae.*

(2) *Sewage Bacteria proper.*
Proteus: Pr. vulgaris.
Anaerobic spore-bearing bacilli: *Cl. sporogenes.*

Studying about 800 strains of bacteria isolated from lakes and streams in the English Lake District, all but one of which were subject to both human and animal pollution, Taylor (1912*b*) found that about 95 per cent. were Gram-negative rods, about 4 per cent. Gram-positive spore-forming rods, and less than 1 per cent. cocci. A large proportion of the organisms were chromogenic; growth on ordinary media was often very poor, and biochemical activity was relatively slight. Taylor points out that these characters differ from those commonly found in soil bacteria; he maintains, in effect, that there is a natural water flora distinct from the organisms washed in with soil.

(For the classification of water organisms see Fuller and Johnson 1899, Boyce and Hill 1900, Jordan 1903, Cornwall 1914.)

Factors determining the Kinds and Numbers of Bacteria in Water

A. Type of Water. Waters may be divided according to their source into (1) surface and (2) deep waters. The former comprise all those that are found on or near the surface of the earth, and that have not been filtered through any considerable thickness of soil; the latter comprise those which, in order to reach the underground stratum that they occupy, have percolated, often for several hundred feet, through porous layers of soil. Since surface waters are frequently exposed to contamination from dust, soil, sewage, factory wastes, and other decomposing organic matter, they may contain large numbers of bacteria, many of which are of intestinal origin. Deep waters, on the other hand, are generally pure, having had most of their surface contaminants filtered off on their downward passage through the soil; and, though it is not unusual to find bacteria in larger numbers than one would expect, these are generally of a harmless type.

(1) Surface Waters.

(*a*) *Rain.*—In falling to the earth, the raindrops come into contact with particles of suspended dust, and carry these down with them. The more dust there is in the atmosphere, the greater is the bacterial count. The figures in Table 195 have been recorded at the Montsouris Observatory at Paris.

From a comparison of these two columns, it will be seen that on the whole the greatest numbers of organisms in rain are found during the dusty months of June, July, August, and September. Montsouris is situated on the outskirts of Paris; in the centre of the city the air was found to contain 6,040 bacteria per cubic metre, and the rain 19,000 bacteria per litre.

TABLE 195

	Bacteria per cubic metre of Air at Montsouris Average for 10 Years	Bacteria per litre of Rain Water at Montsouris. Average for 2 Years.
January	160	8,000
February	145	1,320
March	225	2,920
April	310	2,140
May	305	2,440
June	355	5,600
July	465	5,600
August	455	8,300
September	310	5,770
October	190	3,220
November	195	3,250
December	165	4,330
	Mean 275	Mean 5,300

In the open country the organisms may not exceed 10 or 20 organisms per litre of rain.

(*b*) *Snow.*—This tends to be less pure than rain, probably because the snow-flakes have a greater surface on which to collect suspended particles in the atmosphere ; and also because their low temperature conduces to the survival of bacteria. In snow situated on the tops of high mountains, where it will be remembered that Pasteur found the air to be practically sterile, there are hardly any organisms.

(*c*) *Hail.*—Curiously enough, hail contains more bacteria than either rain or snow. Belli (1902) examined hail that fell in Padua during July, 1901, and found no fewer than 140,000 organisms per litre. Examination of the bacteria showed that they belonged to nine different types. During the formation of hail, it seems probable that rapidly ascending currents of air carry the raindrops up into a region of the atmosphere where they are solidified ; falling down they are melted, and again swept upwards and frozen. After they have been frozen and thawed a number of times the hailstones are thrown out on the periphery of the storm centre and finally come to earth (Mason 1902). It is suggested that the air currents carry up to the cloud region quantities of dust, which is thus incorporated in the hail. It is difficult to explain otherwise the presence in it of vegetable cells, and of fluorescent and soil bacteria.

(*d*) *Ice.*—The number of organisms in ice depends on the nature of the water from which the ice is formed. With the exception of the ice of glaciers, it is generally impure. Its low temperature is favourable to the survival of most bacteria ; hence the self-purification that occurs in waters on storage occurs hardly at all, or very slowly, in ice. For a short review on the bacteriology of ice, see Jensen (1943).

(*e*) *Shallow Wells.*—If protected from contamination in the immediate vicinity by brick sides, and if provided with a pump, shallow wells may contain relatively few bacteria ; but the water of an open draw-well, subject to the influx of dust and of surface washings, is generally very impure. In an examination of over 50 shallow wells, Savage (1906) found that the gelatin count at 20° C. varied from 100 organisms to 20,000 or more per ml. ; and the agar count at 37° C. from less than 10 to over 100 per ml.

(*f*) *Upland Surface Waters.*—If derived from open moorland that is protected from human and animal excretions, these waters are relatively pure. Most of the organisms they contain belong to the soil group of bacteria. The agar count is generally low, not more than 10 or 20 per ml. The gelatin count is fairly high, up to 1,000 per ml.; after heavy rainfall it may rise to several thousands.

(*g*) *Rivers.*—In most countries rivers are heavily contaminated, and contain, not only the natural and the soil bacteria, but large numbers of organisms derived from sewage. In the raw Thames water taken at Hampton during 1906–11 Houston (1913) found the average number of colonies per ml. on gelatin to be 4,310, and on agar 368; typical *Bact. coli* was found in 49·3 per cent. of samples of 0·1 ml. and in 99·2 per cent. of samples of 100 ml.

(*h*) *Lake Waters.*—Owing to the natural storage of water in lakes, there is a continuous process of self-purification occurring; hence lake water is purer than the streams that feed it. Water taken from the middle of the lake contains fewer organisms than that taken near the shore. Lake Geneva contains as many as 150,000 bacteria per ml. near the shore, but further out as few as 38 (Marshall 1921). In Lake Windermere Taylor (1940) found that within 200 metres of the entry of the main river the plate count was 200 times less than at the mouth of the river itself.

(*i*) *Sea Water.*—The number of bacteria in sea water is generally less than in fresh. Russell (1892) found that the water taken near the coast in the Gulf of Naples contained 70,000 organisms per ml., but 4 kilometres out there were only 57. In deep sea the bacteria seem to be distributed evenly, there being almost as many near the bottom as on the surface. The ooze on the bed of the ocean is very rich in bacteria; Russell found 20,000 organisms per ml. Fresh sea water is said to destroy sewage bacteria fairly rapidly (ZoBell 1936), though in estuaries they may remain alive for a considerable time. Part of the destructive action may be due to the activity of protozoa, copepods, and various other lower animal forms (Waksman and Hotchkiss 1937).

(*j*) *Mineral Springs.*—These tend to be relatively pure, and most of the organisms found in water derived from them come from imperfectly sterilized bottles (Duhot and Hutin 1933). The reaction of these waters varies considerably. Bance and Caillon (1929) give the following figures: Badoit pH 6·1, Vals pH 6·05–6·4, Vichy pH 6·4–6·9, Contrexéville pH 6·9–7·0, Vittel pH 7·1, Rubinat pH 7·3–7·6.

(2) DEEP WATERS.—Some of the purest waters that we know come from deep wells and springs. Fifteen driven wells in the neighbourhood of Boston, Mass., contained an average of only 18 colonies per ml. (Prescott and Winslow 1913). In a period of 7 consecutive years, out of 1,565 samples of waters from the deep wells in Kent, Houston found *Bact. coli* in only 5·7 per cent., even when 100 ml. were examined. The purity of springs depends mainly on their source and surroundings. In waters which have percolated through thick strata, the flora differs from that in surface waters; the organisms develop slowly at room temperature, there are few liquefying colonies, and chromogenic organisms are relatively numerous (Prescott and Winslow 1913).

B. Nutrition.—The amount of available food supply is probably the most important factor of all in determining the number of bacteria in a given water. When organic matter is plentiful, organisms abound; when it is scarce they are few, and tend to die out.

C. Temperature.—The effect of temperature varies with the amount of organic matter present. A rise of temperature in a water containing an ample food supply for the bacteria causes them to multiply rapidly: but when the organic matter is small in quantity, a rise in temperature has the reverse effect; this is probably due to early exhaustion of the food supply, and the consequent diminution in rate of multiplication of the bacteria.

A low temperature, independent of the amount of organic matter present, favours the survival, though not the multiplication, of bacteria. Houston (1913) added typhoid bacilli to raw Thames water, and maintained the samples at temperatures varying from 0° C. to 37° C. The initial number added was 103,328 per ml. of water. Table 196 shows how much more rapid was the death of the organisms at 37° C. than at freezing-point. Hamilton (1935), who made observations on the Whangpoo river, found that in the short run from Shanghai to Woosung there was a diminution of 16 per cent. in the colon bacteria during the winter months, and of no less than 97–99 per cent. during the summer months.

TABLE 196

INFLUENCE OF TEMPERATURE. (Houston 1913.)

Degrees.	No. of Bacilli per ml. surviving after weeks.								
	1.	2.	3.	4.	5.	6.	7.	8.	9.
0° C. . .	47,766	980	65	34	3	3	2	1	0·0
5° C. . .	14,894	26	6	3	0·3	0·1	0·0	—	—
10° C. . .	69	14	3	0·3	0·0	—	—	—	—
18° C. . .	39	3	0·4	0·0	—	—	—	—	—
27° C. . .	19	0·1	0·0	—	—	—	—	—	—
37° C. . .	5	0·0	—	—	—	—	—	—	—

D. Light.—It has been asserted that the ultra-violet rays of the sun play an effective part in destroying micro-organisms in water. Procaccini (1893) placed drain water, from which the coarser particles had been removed, in glass cylinders 50 cm. deep, and exposed them for 6 hours to the Italian sun in June. A control cylinder was protected from the light (Table 197).

TABLE 197

	Insolated Cylinder. Bacteria per ml.	Darkened Cylinder. Bacteria per ml.
Before Exposure:		
Surface	4,900	4,900
Middle	4,510	4,510
Bottom	6,781	6,781
After Exposure:		
Surface	0	7,261
Middle	2	9,051
Bottom	8	12,591

The water in the exposed cylinder was practically sterilized; that in the protected cylinder contained rather more organisms than at the start of the experiment.

It would appear that under laboratory conditions the actinic rays of the sun may exert a bactericidal effect. In nature, however, the conditions are altered. One of the main factors hindering the rays is the opacity of the water, which prevents their penetration for more than a short distance. Even in clear water, it is doubtful if they are active for more than a distance of 5 feet from the surface. Another factor is the movement of the water, which may prevent any single organism from being subjected to the rays for a sufficient time to kill it. In this country it is probable that sunlight has little, if any, effect in diminishing the bacterial content of waters. Ultra-violet rays generated from a mercury vapour lamp have, however, been used in the artificial purification of water (Thresh and Beale 1910, Foulds 1911); for this purpose it is essential for the water to be exposed in shallow layers.

E. Acidity.—Winslow and Lochridge (1906) showed that *Bact. coli* in tap water was destroyed by 0·0123 normal hydrochloric acid in 40 minutes. This corresponded to 12·8 parts per million of dissociated hydrogen. Organic acids were likewise bactericidal, but usually in a higher concentration; their effect appeared to be due, not only to the dissociated hydrogen, but also to the undissociated molecule or to the anion (see Chapter 5).

Many natural waters have an acid reaction. In the moorland streams this acidity is due chiefly to the presence of peaty acids—the so-called humic and ulmic acids. Though it is difficult to estimate the effect of acidity on the destruction of bacteria and on the inhibition of their growth, there is little doubt that it does play a considerable part in purifying some waters.

F. Salinity.—The antiseptic and disinfectant action of salts has already been described in the chapter on Disinfection. It is not necessary here to do more than remind the reader of the inimical effects on bacterial growth and survival that certain salts may exercise. It is probably this factor that accounts for the difference both in the numbers and in the types of bacteria found in sea water.

G. Dissolved Oxygen.—Whipple and Mayer (1906) found that *Salm. typhi* and *Bact. coli* remained viable in sterile water containing dissolved oxygen much longer than in water kept under anaerobic conditions. Thus, in one experiment, *Salm. typhi* survived in filtered tap water exposed to the air at room temperature for nearly 2 months, but died in 4 days in an atmosphere of hydrogen. They suggest that this may partly explain why this organism dies more rapidly in polluted than in pure water, and why it survives for a shorter time in summer than in winter. The importance of oxygen in favouring the growth and survival of aerobic and facultative anaerobic organisms has also been stressed by Müller (1912). Taylor (1940), on the other hand, was unable to demonstrate any close relationship between the amount of dissolved oxygen in lake water and the bacterial content; but other factors may possibly have obscured the effect.

H. Protozoal Content.—Huntemüller (1905) showed that flagellates contribute actively to the extermination of bacteria in water. River water naturally polluted by bacteria, or suspensions of *Salm. typhi*, could be cleared in 4 days if flagellates—*Bodo saltans* or *Bodo ovatus*—were added (see also Kyriasides 1931). Stokvis and Swellengrebel (1911) demonstrated a similar action by infusoria—*Colpoda cucullus*. The bacterial destruction was preceded by a rise in the number of protozoa in the water, and was probably due to active ingestion, though this was not demon-

strated conclusively. Aerobic conditions and a temperature between 10° and 30° C. were essential. König (quoted from Thresh and Beale 1925) found that in 1 ml. of water from a well at the Hygienic Institute of Munich, 21 million added typhoid bacilli perished in 24 hours. This he attributed to the action of protozoa. In pure water the death rate in this time was trifling. Increasing attention is being paid to the action of predatory plankton in the self-purification of naturally polluted waters (Butterfield *et al.* 1931, Hoskins 1935). By keeping the bacterial population below the saturation point, it is suggested that the plankton favours the continuous multiplication of bacteria in the water, which results in its turn in a progressive oxidation of organic matter.

I. Rainfall.—The effect of rainfall on the bacterial content of a water is complicated. Rain falling after a drought washes large numbers of soil organisms into the water, and hence increases the numbers of bacteria. If the rain continues for some days, relatively few organisms may be carried in during the later period of rainfall so that the stream is diluted with water purer than its own; its bacterial content therefore decreases. Again, the nature of the water is of importance. A relatively pure stream may be contaminated by rain; an impure stream may be benefited by dilution. As a rule, rivers and upland surface waters contain their greatest numbers of bacteria after heavy rainfall. In Lake Windermere Taylor (1940) observed a close association between the periodic fluctuations in bacterial content and the amount of rain that had fallen in the drainage area during the previous week.

J. Season.—The monthly variation in the bacterial content of waters depends chiefly on the temperature and the rainfall. In this country, the highest counts are generally found in the winter months, when the temperature is low and the rainfall greatest. Rivers show more variation than upland surface waters (Tables 198 and 199, pp. 2018 and 2019). The Thames in winter is swollen by heavy rain, and the quality deteriorates in consequence of the scouring effect over the whole drainage area. In summer many sources of pollution have dried up, and much of the water in the river is virtually stored or filtered water, derived from underground

TABLE 198

RAW THAMES WATER AT HAMPTON. (Houston 1913.)

Month.	Average No. of Colonies per ml. on Gelatin at 20°–22° C. in 3 days.		
	1906.	1907.	1908.
January	2,075	5,246	13,454
February	1,679	3,625	3,627
March	1,161	1,590	2,646
April	277	1,803	6,177
May	1,064	961	2,185
June	382	1,366	1,767
July	952	493	1,107
August	727	436	1,127
September	450	575	1,327
October	439	1,658	1,853
November	2,580	4,902	1,187
December	2,943	6,710	2,188

sources of supply; hence the bacterial content falls (Houston 1917). In upland surface waters, a rise in the bacterial content is not infrequent during July and August; the explanation of this is not clear, but it seems not unlikely that it is the result of dust and soil washings carried in by the summer rains (see Table 199).

TABLE 199

RESERVOIR IN PENNINES. UPLAND SURFACE WATER. MILLSTONE GRIT.

Month.	Average No. of Colonies per ml. on Gelatin at 20°–22° C. in 3 days.		
	1921.	1922.	1923.
January	107	176	68
February	62	65	51
March	25	107	33
April	13	26	14
May	17	1	57
June	80	64	54
July	48	115	60
August	105	86	52
September	30	12	91
October	11	20	13
November	21	89	38
December	154	39	—

In countries in which the water supply is augumented by melting snows, the bacterial content rises considerably in spring time. Oslo obtains its water supply from a lake about 160 metres above sea level. During most of the year, Schmelck (1888) found that the number of organisms per ml. was 10 to 60, rising up to 200 after heavy rain. But in the spring, when the snow was melting, the organisms increased to as many as 2,500 per ml. It is probable that this increase was due to the large amount of earth and detritus which was brought down by the glacial streams.

K. Storage.—The simple storage of water in a reservoir suffices to decrease its bacterial content enormously. Houston (1913) found that after only 15 hours' storage the New River water showed a reduction in the agar count of 40 per cent. He maintains that storage acts in three ways: (1) Sedimentation: the organisms adherent to particles of suspended matter, and those in clumps or zoogloeæ, sink to the bottom, leaving the supernatant water purer. (2) Equalization: this factor comes into play only when several waters of different qualities are collected into one reservoir. It ensures a thorough mixing of the different waters, and prevents the excess distribution of a bad supply on any one day. Even in a river, the water may not be homogeneous; samples taken from one side may be different in their bacterial content from those taken near the other side (see Table 201, p. 2021). In a reservoir, natural or artificial, homogeneity is attained. (3) Devitalization: the organisms die in large numbers, probably from lack of food supply, and ingestion by protozoa. Houston added cholera vibrios to raw river water, and found that after 1 week's storage their numbers had been reduced by 99·9 per cent.; after 3 weeks they could not be isolated even from 100 ml. of water. Table 200 shows the effect of storage on the London water.

TABLE 200

EFFECT OF STORAGE. 1907–08. AVERAGE RESULTS. (Houston 1913.)
Bacteria per ml.

	Gelatin, 20°–22° C.	Agar, 37°C.	No. of Samples with *Bact. coli* in 0·01 ml.
River Thames before storage	4,465	280	10·1%
River Thames after 15 days' storage at Chelsea	208	44	1·1%
Reduction	95·3%	84·3%	89·1%

The reduction occurs not only in pathogenic organisms and *Bact. coli*, but in organisms of all sorts, though not always equally. Houston states that even 1 week's storage would be more efficacious, in reducing the initial numbers of typhoid bacilli or cholera vibrios in a water, than sand filtration.

It is probably owing to storage that lake waters are so much purer than the streams that feed them. Some rivers with a very low gradient may offer conditions suitable for sedimentation.

L. Filtration.—Natural filtration occurs on a large scale, resulting in the accumulation of the underground deposits of water that are tapped by deep wells and main springs. Its efficacy in the removal of bacteria depends on the nature of the soil, and the depth of the strata penetrated. In loose, porous soils a greater depth must be traversed to ensure the same degree of purification that is attained by filtration through a more compact soil. Evidence suggests that, in a soil of moderate density, the greater part of the bacteria are removed in the first 10 or 15 feet. This is the reason why deep well water is so pure.

Artificially, sand filtration is used to remove bacteria from water in order to render it potable. Houston (1913) finds that this process, which in the case of the London waters follows storage for 30 days, removes 98 per cent. of the residual bacteria.

M. Other Factors.—It is known that certain rivers have a bactericidal effect on some of the intestinal organisms. Thus Arloing and Sempé (1924) state that the water of the Saône inhibits the growth of *Bact. coli* but not of *Salm. typhi*. The water of the Isère inhibits the growth of *Salm. paratyphi A*; the Rhône *Salm. typhi*; and the sea at Havre *Shigella shigæ* (Arloing and Chavanne 1925, Arloing and Sempé 1926). In India the water of the Ganges is said to destroy the cholera vibrio (see Khan 1930). Arloing and Sempé (1926) and Bujanowski (1929) have advanced some evidence to suggest that this inhibitory, and often highly specific action, is dependent on the presence in the water of a transmissible lytic agent, similar to that described by Twort and by d'Herelle. If this work is confirmed, it will be an interesting addition to our knowledge of the means by which waters undergo natural purification; and it may serve to explain why certain regions are relatively immune to water-borne diseases—though this seems unlikely.

Self-Purification of Rivers.

From what has already been said, it is clear that the number of bacteria in a particular sample of water is determined by several factors, the precise effect of which it is difficult to assess. It is also clear that such factors as absence of organic matter, the presence of numerous protozoa, a high acidity, and the opportunity

for sedimentation, are able to bring about a considerable reduction in the bacterial flora. It is not surprising, therefore, that rivers that have been heavily polluted by sewage may regain their natural purity after flowing for some distance. Jordan's (1900) observations on the Illinois river are of interest. The portion of the river that he examined was between Morris and Ottawa. Nine miles above Morris two tributaries unite, the relatively pure Kankakee, and the Desplaines, laden with Chicago sewage. During its flow of 24 miles from Morris to Ottawa the river improved in purity in spite of the absence of dilution with fresh water. The observations were made in October and November when there was neither sun nor rain (Table 201).

TABLE 201

	Distance from Morris.	No. of Colonies per ml.			No. of Hourly Analyses.
		Right Bank.	Middle.	Left Bank.	
Upper station, Morris .	—	261,000	204,000	29,000	3
Middle station, Seneca .	12 miles (24 hours)	100,000	49,000	35,000	2
Lower station, Ottawa .	24 miles (48 hours)	11,500	10,700	13,500	2

The extraordinary degree of purification that occurred was accounted for partly by the slow movement of the river, which permitted sedimentation to occur, and partly by the absence of sufficient food-supply to support the large number of contaminating organisms. Jordan found between Morris and Ottawa a decrease in the albuminoid ammonia of 0·382 to 0·049 parts per million, and in the oxygen consumed of 2·3 to 2. This diminution was probably due to bacterial action.

There is no space to discuss more fully the self-purification of rivers. Enough has been said to indicate the most important factors controlling it. Moreover the importance of each factor varies with different rivers. The reader is referred to a study of the self-purification of the Spree below Berlin by Frank (1888), of the Danube below Vienna by Brezina (1906), and of the Whangpoo by Hamilton (1935).

Bacteriological Analysis

We do not propose to give a detailed description of the bacteriological analysis of water, since the Ministry of Health (Report 1939*a*) in this country and the American Public Health Association (1936*b*) in the United States have each described standard methods for its performance, to which reference should be made by those desirous of further knowledge. Great attention has to be paid to the sampling of the water (for details see Report 1939*a*), and to the technical performance of the various procedures involved (for error of these see Wilson *et al.* 1935).

Plate Count.—In general, the analysis consists in an enumeration of the organisms or groups of organisms capable of forming colonies on a standard nutrient agar medium incubated aerobically at 20°–22° C. for 3 days or at 37° C. for 2 days. Since not all the organisms in water are viable ; since many viable organisms, such as those of the anaerobic and nitrifying groups, do not develop under the particular conditions provided ; and since some of the organisms occur in groups which give rise only to a single colony—it is clear that the colony count corresponds not to the total number of organisms or even to the number of viable organisms, but only

to the number of bacterial units that are able to multiply under the nutritional, respiratory, and temperature conditions supplied. For this reason it should be reported either as the " number of colonies developing per ml.," or more simply as the " plate count per ml."

Coliform Count.—Besides a general bacterial enumeration, an attempt is made to estimate the number of coliform bacteria in the water, and often to ascertain the proportions of the various types of these organisms. The ideal method of doing this would be a direct plate count on a differential medium. So far, however, no medium has been devised that will differentiate with certainty between coliform and non-coliform organisms, or between the different types of coliform bacilli. Partial success in these respects has been claimed by Tonney and Noble (1930, 1931*a*, *b*, 1932*a*, *b*) for their ferrocyanide citrate agar medium, by Gehm and Heukelekian (1935) for eosin methylene blue agar, and by Littman and Stark (1938) for citrate ricinoleate agar.

The usual technique for estimating coliform bacilli is by the dilution method in a liquid medium, which allows the observation of gas production—an important property serving to distinguish coliform from most non-coliform bacilli. As has already been pointed out (Chapter 4), the dilution method is subject to a big sampling error. The results depend not only on the number of dilutions prepared and on the number of tubes of each dilution inoculated, but also on the number of organisms present in each unit volume of the original water. Hence attempts have been made to devise the most economical method of carrying out the examination consistent with reasonable accuracy (Halvorson and Ziegler 1933*a*, *b*, Swaroop 1940, 1941). Even greater effort has been expended in endeavouring to convert the results obtained into terms of the probable number of organisms present (see Greenwood and Yule 1917, McCrady 1918, Hoskins 1934, Matuszewski and Supińska 1937, Haldane 1939, Pomeroy 1940, Buchanan-Wollaston 1941). No perfect medium has yet been devised that will enable all coliform organisms to develop and produce gas, while suppressing the growth, or at least the gas formation, of other organisms.

British practice favours MacConkey's bile salt lactose medium, but in the United States other media, such as lactose broth (Report 1936*b*), brilliant green lactose bile broth (Jordan 1927), fuchsin lactose broth (Ritter 1932, 1938), methylene blue brom cresol purple broth (Dominick and Lauter 1929), crystal violet lactose broth (Salle 1930), formate ricinoleate broth (Stark and England 1935), gentian violet lactose bile broth (Kessler and Swenarton 1927), the so-called " E.C. " medium (Perry and Hajna 1944), and lauryl sulphate tryptose broth (Mallmann and Darby 1941), have been recommended. It is impossible to say which of these media is the best, since reports on comparative trials are at variance (see Shunk 1935, Raghavachari and Iyer 1936, Farrell 1937, Atkinson and Wood 1938*a*). The results may well depend on the bacterial flora of the water under examination. Generally speaking, there is an advantage in not having to depend on dyes to suppress the growth of non-coliform organisms, since standardization of the medium is difficult to achieve.

In this country a series of MacConkey broths are inoculated with falling quantities of water, namely one 50 ml. quantity, five 10 ml., five 1 ml., and, if necessary, five 0·1 ml. quantities, incubated at 37° C. for 2 days, and the number of tubes showing acid and gas noted. The results are reported in terms of the probable numbers of coliform bacilli per 100 ml. (see Report 1939*a*). Since acid and gas production may be due to certain aerobic spore-bearers (see Greer 1928, Porter *et al.*

1935), or to the synergic action of two different types of bacteria, one of which attacks lactose and the other of which produces acid and gas from the glucose so formed (see Atkinson and Wood 1938*b*), the count is best referred to as the "presumptive coliform count." In Great Britain, where MacConkey broth is used, false positive results from these causes are uncommon in water examination (see Bardsley 1938*a*); but in the United States, where lactose broth free from bile salt is the standard medium, a varying proportion of the presumptive positive tests are not "confirmed" on further investigation.

Differential Coliform Count.—Not infrequently it is desirable to ascertain the types of coliform organisms present. For this purpose it is usual to plate out on to MacConkey agar two or three of the positive tubes inoculated with the smallest quantities of water, to incubate for 24 hours, to pick off 2 or 3 lactose-fermenting colonies from each plate, and to test these in a series of media capable of differentiating between organisms of the coli, intermediate, and aerogenes-cloacæ types (see Chapter 28). This method, besides being tedious, suffers from two main objections. Firstly, if one type of organism is present in the original water in much smaller numbers than another type, it is liable to be diluted out during the preparation of the dilutions, so that plates inoculated from the positive tubes seeded with the smallest quantities of water will yield a culture of the commoner organism only. Secondly, even if the two types are present in approximately equal numbers, one type is liable to outgrow the other, so that again a pure, or almost pure, culture of one type develops on plating.

To obviate these errors, a method has been suggested (Wilson *et al.* 1935) which consists in subculturing *all* MacConkey broths showing acid and gas (1) into a fresh series of MacConkey broths, which are incubated at 43°–45° C. in a water-bath, and (2) into tubes of Koser's citrate, which are incubated at 37° C. The production of gas in MacConkey broth—but not necessarily in other media—at 44° C. seems to be characteristic of *Bact. coli* of fæcal origin, while the ability to grow in Koser's citrate is distinctive, in the coliform group, of the intermediate and aerogenes-cloacæ types. By making use of probability tables it is thus possible to obtain within 3 or 4 days a quantitative estimate of the organisms belonging to the coliform, the fæcal coli, and the intermediate-aerogenes-cloacæ groups. In this method, though the different types may be present in very unequal proportions in the fermented tubes, transference to selective liquid media will enable growth of even minimal numbers to occur.

The value of the 44° C. MacConkey test for picking out *Bact. coli* of fæcal origin has now been abundantly confirmed (see p. 664); though in Madras and Singapore some strains of *aerogenes* type have been found capable of producing gas at this temperature (Raghavachari and Iyer 1939*a*, Boizot 1941). The citrate test is less specific for members of the intermediate-aerogenes-cloacæ group when used for direct inoculation from a fermented 37° C. MacConkey broth culture, owing to the ability of some late-lactose and non-lactose fermenters and even *Bact. coli* to grow in this medium (see Bardsley 1938*a*, Raven *et al.* 1940, Ferramola 1940). In practice, however, this is not usually a serious drawback, since most false positives in citrate occur with the more polluted waters, which would be condemned in any case on the presumptive coliform or the fæcal coli count.

The selective liquid media method has now been adopted officially by the Ministry of Health (Report 1939*a*) for the differential coliform test in the bacteriological examination of waters. In the extensive trials during the second world

war, it proved to be reliable, and to be economical in both time and medium. The specificity of the 44° C. MacConkey test for *Bact. coli* in this country has only once been questioned (Harding 1940), and on investigation the criticism proved to be unfounded. It may, however, be noted that Taylor (1945), working in a particular locality, has reported gas production in MacConkey's medium at 44° C. by as many as 15 per cent. of *Bact. aerogenes* strains.

Classification of Coliform Organisms in Water.—In Chapter 28 we have discussed the classification of lactose-fermenting coliform bacilli according to the general principles of systematic bacteriology. In water analysis it is of greater advantage to classify these organisms in relation to their habitat, paying particular attention to their excretal or non-excretal origin. Such a classification is given in Table 202.

TABLE 202

CLASSIFICATION OF COLIFORM STRAINS MET WITH IN GREAT BRITAIN

Type.	M.R.	V.P.	Growth in Citrate	Indole.	Gas in MacConkey at 44° C.	Gelatin Liquefaction, 7 days.	Probable Habitat.
Bact. coli, Type I, fæcal	+	−	−	+	+	−	Human and animal intestine.
Bact. coli, Type II. .	+	−	−	−	−	−	Doubtful; possibly partly intestinal.
Intermediate, Type I .	+	−	+	−	−	−	Mainly soil.
Intermediate, Type II	+	−	+	+	−	−	Mainly soil.
Bact. aerogenes, Type I	−	+	+	−	−	−	Mainly vegetation.
Bact. aerogenes, Type II	−	+	+	+	−	−	Mainly vegetation.
Bact. cloacæ . . .	−	+	+	−	−	+	Mainly vegetation.
Irregular, Type I . .	+	−	−	+	−	−	Human and animal intestine.
Irregular, Type II. .	+	−	−	−	+	−	Doubtful.
Irregular, other types	Reactions variable.						Doubtful.

It will be seen that *Bact. coli* Type I, Irregular Type I, and possibly some of *Bact. coli* Type II (see Bardsley 1938*a*, Stuart *et al.*, 1942) appear to find their natural habitat in the mammalian intestine. It is true that strains belonging to the intermediate-aerogenes-cloacæ (I.A.C.) group are often found in fæces, but as a rule they are present in only very small numbers. Their natural habitat is still unknown (see Taylor 1942*a*), but the great mass of evidence suggests that it is not primarily intestinal. Since Irregular Type II, which produces gas in MacConkey at 44° C., is very uncommon in fæces, it follows that a positive 44° C.

MacConkey test is practically diagnostic of fæcal coli. The fact that neither *Bact. coli* Type II nor Irregular Type I produces gas in MacConkey at 44° C. is of little practical importance, since these organisms constitute only a very small proportion of the fæcal coliform flora (Bardsley 1938*a*).

Examination for Fæcal Streptococci, Cl. welchii, and Pathogenic Organisms.—The plate and coliform counts constitute the usual water analysis. Sometimes, however, further information is required, and search may have to be made for special organisms.

In examining water for fæcal streptococci advantage is taken of the partial heat resistance of these organisms, and of their ability to form acid in MacConkey's medium and to grow in the presence of a concentration of potassium tellurite sufficient to prove inhibitory to most coliform organisms (see Report 1939*a*).

The search for *Cl. welchii* is conducted either by anaerobic incubation of litmus milk after preliminary heating to 80° C. for 10 minutes to destroy non-sporing organisms, or by use of W. J. Wilson's (1928) glucose sulphite iron agar medium.

Typhoid and paratyphoid bacilli are difficult to demonstrate in water, partly because of technical difficulties, and partly because, unless the contamination of the water is continual, these organisms will have died out by the time suspicion of their presence is aroused. Far and away the most satisfactory method is the use of the bismuth sulphite brilliant green medium devised by W. J. Wilson and Blair (1931) (see also W. J. Wilson 1938). When the difficulties of making this medium have been overcome, the results are highly successful, and typhoid and paratyphoid bacilli can be isolated even from crude sewage (see Houston 1930).

In the demonstration of *V. cholerœ* advantage may be taken of its rapid growth in alkaline peptone water under aerobic conditions, and of the various differential media available (see Chapters 22 and 63).

Interpretation of the Bacteriological Analysis.—Before attempting to give an opinion on the results of a bacteriological analysis, it is essential to gather particulars of the nature of the water, the method by which it was collected, the time of collection, and the amount of recent rainfall. It is sound practice, though not always possible, for the bacteriologist to make a topographical survey of the gathering ground, so as to ascertain the extent and the kind of pollution to which it is subject. If he is unable to do this personally, he should consult a map in which the source of the water and the immediate environment are indicated. Particular care must be given to the mode of collection of the sample; otherwise contamination, especially with coliform organisms, may disturb the interpretation of the results (for instructions, see Report 1939*a*).

The whole aim of the bacteriological analysis of water is to find evidence of excretal pollution. Since we can rarely isolate directly specific pathogenic organisms, such as *Salm. typhi*, we resort to estimating first of all the number of living bacteria of all sorts in the water, and secondly the number of living bacteria of intestinal origin. The greater the number of bacteria, the greater, presumably, is the amount of decomposing organic matter. The more bacteria of intestinal origin there are, the more likely are pathogenic species to be amongst them. Our evidence therefore is circumstantial, and is frequently open to doubt in its interpretation.

Unfortunately we cannot lay down absolute standards for all waters; but we can lay down a standard for any one water. This is possible by frequent and regularly repeated examinations, which teach us the range of normal variation.

We become acquainted, in fact, with the bacterial character of the water. Any marked deviation from the norm is at once regarded with suspicion. On a water which we examine for the first time and for which we have no absolute standards, it is often difficult to express more than a tentative opinion. A high agar count at 22° C., for example, may be significant or it may not; and though the sanitary survey may assist us on this point, it may not be till several examinations under different conditions have been made that we can offer a definite opinion.

The agar count at 22° C. gives us information on the amount of decomposing organic matter in the water available for bacterial nutrition. Most bacteria that develop at 20° C. are non-pathogenic to human beings, and it might therefore be thought that their number was immaterial. On the other hand they afford some indication of the amount of extraneous organic matter available for bacterial nutrition that has gained access to the water from various sources. On general grounds the greater the amount of organic matter present, the more likely is the water to be contaminated with parasitic and potentially pathogenic organisms. Occasionally in unpolluted deep well waters there may be a high agar count at 22° C., running into thousands or tens of thousands per ml. This is found, as a rule, to result from the presence of a single species of organism that has apparently gained access from the air and multiplied abundantly. The colonies develop slowly and are often chromogenic. Such a finding can usually be ignored

The agar count at 37° C. is a more important index of dangerous pollution. Most of the natural and harmless bacteria in water do not grow readily at 37° C.; the organisms developing at this temperature are chiefly of soil, sewage, or intestinal origin. Hence our standards must be more stringent than at 22° C. A high 37° C. agar count is often sufficient to condemn a water, though there are a number of exceptions to this rule. For example, recently sunk wells and bores are liable for some time to give an unduly high count, which later decreases as the well settles. In upland surface waters the 37° C. agar count may be very high, sometimes exceeding 1,000 per ml., even in the absence of excretal pollution. During the summer months the count may rise in such waters, owing partly tothe access, of dust and organic matter to the water, and partly to the increase in te mperature both of which favour bacterial multiplication. Waters stored under certain conditions, as in metal tanks, may have a 37° C. agar count in the thousands, though no coliform bacilli may be demonstrable. The 37° C. agar count is of particular value in the control of filtration. With slow sand filters the count on the filtered water should show a 95–98 per cent. reduction on that of the raw water. A rise in the colony count is the usual signal of defect in the filter beds, demanding instant attention.

It is generally found that in a pure water about ten times as many organisms develop at 22° C. as at 37° C.; in a polluted water this ratio is often less. Some workers place more weight on the value of this ratio than on the individual counts. This, we consider, is a mistake. The ratio may vary within wide limits, quite independently of the amount of fæcal contamination. Table 203 brings out this point. Moreover, in a water treated with chlorine, the number of colonies at 22° C. and 37° C. may be almost equal. This is doubtless accounted for by the susceptibility of the natural water bacteria, and the resistance of the spore-bearing soil bacteria; both counts are lowered absolutely, but the 22° C. far more than the 37° C.

Just as the 37° C. is of greater significance than the 22° C. agar count, so the number of coliform bacilli is a more reliable index of excretal pollution than the

37° C. plate count. It is also easier to lay down an absolute standard for *Bact. coli* than for either of the other two counts. For example, this organism should not exceed 1 per 100 ml. in deep well water, or 5 per 100 ml. in a shallow well or upland surface water. Its presence in greater numbers is strongly suggestive of pollution.

TABLE 203

Modified from Thresh and Beale (1925).

Source of Water.	Colonies per ml.		Ratio of Gelatin to Agar.
	Gelatin 20° C.	Agar 37° C.	
Unpolluted deep well in chalk	61	3	20 : 1
Do.	161	13	12 : 1
Do.	9	3	3 : 1
Unpolluted spring water	51	3	17 : 1
Do.	34	6	6 : 1
Polluted deep well in chalk	84	4	21 : 1
Do.	260	72	4 : 1
Polluted shallow well	1,560	38	41 : 1
Do.	151	4	36 : 1
Polluted river water	6,300	140	45 : 1
Do.	29,600	3,300	9 : 1

A distinction should be drawn between the typical coli I, which is usually of excretal origin, and the other types of coliform bacilli, which are more often derived from other sources (see Table 202). There is no question that coli I—the fæcal coli type—is the most delicate index we have of recent excretal pollution. With regard to the importance of the intermediate and aerogenes-cloacæ types (I.A.C.) opinion is divided. There are those who maintain that, because these organisms are found in fæces—though only in small numbers—their presence cannot be neglected. There are those who draw attention to the fact that organisms of the aerogenes type are often present in infected urine (Hill *et al.* 1929, Burke-Gaffney 1933), and may constitute the dominant type of coliform bacilli in human fæces (Parr 1936). There are others who point out that, if recent excretal pollution has occurred, fæcal coli will undoubtedly be found too, so that the presence of other types in the absence of fæcal coli can generally be ignored.

The interpretation is to some extent affected by the rate at which these different organisms die out after gaining access to water. Here again our data do not provide us with a clear answer.

The vitality of these organisms in water varies with several factors. Houston (1913) found that storage of water for 15 days reduced the number of *Bact. coli* by 80–90 per cent. Gray (1932) and Burke-Gaffney (1933) bring evidence to show that *Bact. coli* dies out more rapidly than *Bact. aerogenes*. Ruchhoft and his colleagues (1933) find that both organisms disappear at about the same rate. Platt (1935) finds that this rate depends to some extent on the temperature, *Bact. aerogenes* surviving longer than *Bact. coli* at 18°–20° C., but not at 0° C. or 37° C. Raghavachari and Iyer (1939*b*) suspend their judgment. Hamilton (1935), in China, finds that in the summer months *Bact. aerogenes* may actually multiply in water under favourable conditions.

On the whole, the evidence suggests that organisms of the I.A.C. group tend to be rather more resistant to environmental conditions than fæcal coli. Their presence in water, in the absence of fæcal coli, may indicate either (1) that non-

polluted dust or soil has gained access to the water; or (2) that excretal contamination has occurred at a time sufficiently remote to permit the disappearance of all fæcal coli organisms; or (3) that the water is subject to a minor degree of excretal contamination heralded, for some reason which is not yet clear, by the appearance of organisms of the I.A.C. group before true fæcal coli; or (4) that a contaminated water has been insufficiently chlorinated; or (5) that the water—and this applies to wells and pumps—is being contaminated from such material as old sacking, leather washers, jute packing, or decaying leaves, in which organisms of the I.A.C. type are actively growing. Which of these explanations is correct, it is usually impossible to say on any one sample of water without further examination.

In this country a high proportion of waters giving a positive presumptive coliform test contain fæcal coli (Bardsley 1934, 1938*a*), and it is therefore often unnecessary to proceed to a differentiation of the coliform types. In the routine control of water supplies from which coliform bacilli are usually absent, the appearance of these organisms demands immediate attention, and differentiation should be carried out in order to obtain a clue to the probable source of pollution. Differentiation is likewise called for when a new supply is under examination, as it is always desirable to learn as much as possible about the flora normally present. In other types of water differentiation is a matter for individual judgment. Generally speaking, there is little point in determining the nature of the types present in a piped water supply if the presumptive coliform count exceeds about 10 per 100 ml., since even if no fæcal coli are found the water will still have to be regarded as probably contaminated. In shallow well waters, however, differentiation may be useful with a coliform count up to about 50 per 100 ml., as the degree of excretal pollution may be so small as to render it unnecessary to condemn the water when, as usually happens, it is being consumed by only a small number of persons. Unfortunately, there is no reliable method of distinguishing coliform organisms of human and animal origin.

In the *tropics* a fairly high proportion of coliform organisms in water are often found to be of the intermediate or aerogenes-cloacæ types. Since careful sanitary surveys have shown that such waters may be free from exposure to excretal contamination, it is clear that reliance on the presumptive coliform count in the tropics will result in the unnecessary condemnation of a number of unpolluted waters. Differentiation will generally be necessary, and attention should be devoted mainly to the numbers of fæcal coli.

In cases where the probable origin of coliform bacilli is doubtful, use may be made of the tests for fæcal streptococci and *Cl. welchii*. Both of these organisms are commonly present in mammalian fæces. If they are found in significant numbers in the water, they may be regarded as indicating that the doubtful coliform bacilli were probably of excretal origin. Their absence, on the contrary, points to the reverse conclusion. It is mainly as an aid to the interpretation of the coliform result that a test for these organisms finds its real value. It must be pointed out, however, that both fæcal streptococci and *Cl. welchii* probably survive longer in water than fæcal coli, so that their presence in the absence of this organism is generally indicative of remote rather than of recent excretal contamination. (For further information on the coliform group of organisms in relation to water supply in the tropics reference should be made to papers by Pawan 1925, 1926, 1931, Raghavachari 1926, Raghavachari and Iyer 1939*a*, *b*, Hicks 1927, Taylor 1927, and Burke-Gaffney 1932, 1933.)

Standards.—It will be obvious that the larger the population is to which the water is distributed, the more stringent must our standards be. The occurrence of typhoid fever in the inmates of a cottage drawing water from a polluted well is unfortunate, but its occurrence in the population of a large town is a catastrophe. The standards that we give here are those suggested by the Ministry of Health (Report 1939*a*), and refer only to piped water supplies. As will be seen, they are based entirely on the results of the coliform test, since this affords the most delicate, the most specific, and at the same time the most generally applicable index of pollution.

Non-chlorinated Piped Water Supplies

		Presumptive Coliform Count per 100 ml.
Class 1.	Highly satisfactory	less than 1
Class 2.	Satisfactory	1–2
Class 3.	Suspicious	3–10
Class 4.	Unsatisfactory	Greater than 10

Owing to the large sampling error of the coliform count, too much attention should not be paid to minor fluctuations in these figures. For this reason the Ministry proposes that throughout the year 50 per cent. of samples should fall into Class 1; that 80 per cent. should not fall below Class 2; and that the remainder should not fall below Class 3. Chlorinated waters ought uniformly to come into Class 1.

For the student's guidance we give two examples of unpiped and unpurified waters destined for consumption by a small population. Though the deep well and main spring water would be safe for a town supply, the second water we quote would have to be purified before its delivery to a large number of consumers would be justified.

Deep Well and Main Spring Waters

Plate count, agar 3 days at 22° C.	10–200 per ml.
Plate count, agar 2 days at 37° C.	1–10 per ml.
Bact. coli fæcal Type I	Less than 1 per 100 ml.
Fæcal streptococci	Less than 1 per 100 ml.
Cl. welchii	Less than 1 per 1,000 ml.

Shallow Well, Land Spring, and Upland Surface Waters

Plate count, agar 3 days at 22° C.	50–500 per ml.
Plate count, agar 2 days at 37° C.	5–30 per ml.
Bact. coli fæcal Type I	Less than 5 per 100 ml.
Fæcal streptococci	Less than 5 per 100 ml.
Cl. welchii	Less than 5 per 1,000 ml.

A filtered river water should conform to the standard of a good shallow well water. If chlorination has been used in addition, then the coli standard should equal that of a deep well water.

The effect of rainfall on the water should be noted carefully. Speaking generally, the less the water is influenced by this factor the better. Though rain in itself contains relatively few bacteria, it may carry in large numbers of undesirable organisms from the soil. A large increase in the number of organisms, especially if attended by a rise in the coli count, should always be regarded with suspicion.

There is often a tendency for more weight to be placed on the results of the

bacteriological examination than is justifiable. If the sanitary survey shows the water to be clearly subject to human excretal pollution, then it is not exaggerating to say that a bacteriological examination is redundant. Even should a reasonably good result be obtained on one sample, there is no doubt that repetition on further samples, taken perhaps after heavy rainfall, will reveal the presence of coliform bacilli in large numbers. Bacteriological examination is a delicate tool suitable for revealing pollution too small in amount or too obscure to be noticeable by the ordinary means of the topographical survey. If the relatively crude method of the survey shows the presence of undoubted contamination, then the delicate methods employed by the bacteriologist are seldom required. It follows that no water exposed to known contamination should ever be reported on favourably by the bacteriologist, regardless of what the laboratory findings may be.

Finally we repeat that judgment on the potability of a water can be given only after a careful weighing of all the evidence available. It must be remembered that the mere absence of evidence indicating fæcal pollution does not necessarily indicate that pollution has not taken place. All it does show is that at the time the sample was examined, there was no detectable evidence of pollution. It is partly for this reason that frequent examinations are desirable.

The interpretation of the bacteriological analysis, particularly on a new supply, is greatly facilitated by a concurrent chemical analysis, which furnishes information on the amount of organic matter in the water. The results obtained by the two methods of examination should be carefully correlated. As a rule, they are in substantial agreement, but discrepancies are occasionally found which show the need for caution in the report, and perhaps for the desirability of a further examnation.

Swimming Bath Waters.—The main organisms found in swimming bath water are aerobic spore-bearers, *Pseudomonas*, *Chromobacterium*, coliform bacilli, *Proteus*, staphylococci of skin and salivary origin, streptococci, *Neisseria*, and *Cl. welchii*. According to Bardsley (1938*b*) most of the coliform bacilli are of excretal origin and consist chiefly of organisms of the coli I type; but members of the I.A.C. group are also present, and in chlorinated waters may actually exceed coli I in number. A little reflection will enable the student to understand why the bacteriological control of swimming bath waters is unsatisfactory. The results vary from one part of the bath to another, and from minute to minute in the same part depending on the numbers and personal cleanliness of the bathers. Though the addition of chlorine to the water to give a residual figure of 0·2–0·5 parts per million should result in a sterile 37° C. agar plate count and in the absence of coliform organisms from 50 ml. of the incoming water (Report 1929), it is impossible to maintain virtual sterility in the bath itself without adding chlorine in such excess as to cause severe irritation to the nasal and conjunctival mucosa of the bathers. Infection, either of nasopharyngeal or of excretal origin, may pass from one person to another with great rapidity, so that no bearable chlorine concentration can be expected to destroy the organisms concerned. In practice it is wise to examine the in-flowing and the out-flowing water, if continuous circulation is being maintained. The standard of the in-flowing water should be that quoted above. For the out-flowing water no standard can be laid down, but if the 37° C. agar plate count exceeds 200 per ml., and the presumptive coliform count exceeds 2 per 100 ml., steps should be taken for more rapid circulation of the water, or for a higher residual chlorine concentration in the water entering the bath.

Water-borne Disease.—The main diseases carried by water are enteric fever, dysentery, and cholera. For a description of these reference should be made to Chapters 63, 69, 70. In this country the incidence of water-borne disease is low compared with some parts of the Continent and with the United States, but is by no means negligible. Between 1911 and 1937 there occurred in England and Wales at least 21 outbreaks of disease conveyed by public water supplies, resulting in 1,237 cases of enteric fever, 2,800 of bacillary dysentery, and 7,439 of gastro-enteritis (Report 1939*b*). In the United States, Gorman and Wolman (1939) were able to collect records of 399 outbreaks during the period 1920 to 1936, affecting 115,645 persons. Among the cases of disease were 12,585 of typhoid fever and 101,603 of diarrhœa. It may be noted that paratyphoid fevers are seldom water-borne. Franklin and Halliday (1937) report one outbreak of paratyphoid A fever in Canada due to contamination of a well water from a resident who had returned to her home suffering from an undiagnosed disease; and there have been 2 or 3 small outbreaks of paratyphoid B fever in this country (see Report 1942, Page 1942). As is apparent from the records just given, diarrhœa and gastro-enteritis figure prominently in water-borne disease. Their nature and causation are not always clear. Some outbreaks may be due to undetected dysentery bacilli, but there seems little doubt that the access of sewage in considerable amount to a water supply may be followed by gastro-enteritis in the absence of any recognized pathogenic organisms (see Kathe and Königshaus 1932, Report 1936*a*, 1937, Pharris *et al.* 1938). Lead poisoning has, of course, to be borne in mind, as well as less common forms of chemical poisoning, such as that described by Campbell (1940) of naphthalene poisoning following the painting of a storage tank.

Infection of the water may occur at the source, during storage, or during distribution. The prevention of water-borne disease demands scrupulous care and supervision of every part of the system, including the health of operatives closely associated with the water itself (see Report 1939*b*).

SHELL FISH

The chief importance of shell fish from a public health point of view is their liability to give rise to enteric fever. In France alone it is estimated that within a period of 15 years more than 100,000 cases of typhoid fever due to the consumption of shell fish occurred, of which 25,000 ended fatally (Belin 1934).

Shell fish become infected by being laid down in polluted water. According to Eyre (1924), about 2 litres of water enter and leave the shell of an oyster every hour. Large volumes of water also pass through mussels every 24 hours. The bacterial flora of these shell fish is largely determined by the nature of the water in which they are immersed. This is often subject to rapid changes. It is quite possible, for example, for mussels to be heavily polluted on an ebb tide and to clean themselves on a flood tide. The only satisfactory way of ensuring their purity is to lay them down for some days in non-polluted, preferably chlorinated, water (see Dodgson 1936).

An attempt is often made to control the suitability of shell fish for human consumption by bacteriological methods. The general principles of such an analysis are similar to those we have just discussed in the examination of water, particular

attention being paid to the numbers of coliform bacilli. The difficulties, however, of obtaining an exact bacterial enumeration are considerably greater with shell fish than with water. The chief reason for this is the extraordinary variation in numbers from one oyster or mussel to another, and the gross irregularity in distribution of organisms within the individual shell fish themselves. There is in this country no standard method of analysis or standard of interpretation laid down for the examination of shell fish, with the result that many workers have evolved their own technique. Since the methods of different workers often differ in major points, we shall refrain from describing any one in detail. Those who are interested may be referred to papers by Eyre (1924, 1933), Dodgson (1928, 1936, 1938), Perry (1928, 1929), Bigger (1934), Beard and Meadowcroft (1935).

The standard adopted by the Fishmongers' Company in this country, based on the recommendations of Andrewes, Hewlett and Eyre (1924), allows approximately 100 coliform bacilli per oyster in 40 per cent. of the oysters sampled. For purified oysters Dodgson (1938) exacts a more rigid standard, namely not more than 50 coliform bacilli in any oyster. The presumptive coliform test, however, may be grossly misleading, as organisms of the aerogenes-cloacæ type may, under favourable conditions, multiply enormously in mussels, barnacles, raw sea water, and sterilized tank water. This difficulty may be overcome by using the 44° C. MacConkey broth test for fæcal coli (Dodgson 1938). Just as with water, so in the control of shell fish, a careful topographical survey to exclude possible sources of pollution is essential; bacteriological examination must remain as an ancillary method to check the results of the sanitary investigation.

SEWAGE

In this country crude sewage contains about 10 million organisms per ml. capable of developing on gelatin at 20° C., and from 1 to 5 million per ml. on agar at 37° C. In America the numbers appear to be lower. There is often a marked rise in the summer months. The organisms making up these numbers are of many different kinds, and vary from one sewage to another. Prominent amongst them are the *Proteus* group, the coliform bacilli, streptococci, anaerobic spore-bearing bacilli, natural water bacteria, and the denitrifying bacilli. *Bact. coli* may number 100,000 per ml., *Streptococcus fæcalis* 1,000 to 10,000 per ml., and *Cl. welchii* 100 to 1,000 per ml. Pathogenic organisms of the typhoid, paratyphoid, and food-poisoning groups can often be found in crude sewage by the use of appropriate selective media. The poliomyelitis virus has also been demonstrated in it (Paul and Trask 1941, 1942). Sewage is an excellent source of a large variety of bacteriophages.

The process of purification is accompanied not so much by a diminution in the numbers of organisms, as by a change in the distribution of different organisms. Thus in the septic tank, the anaerobic liquefying bacteria are prominent; on the contact beds the aerobic liquefying and the denitrifying bacteria gain the upper hand. It is evident that enormous numbers of bacteria must perish in the process; by a comparison of the microscopic and the gelatin counts, Winslow (1905) found that the ratio of the total to viable organisms was about 20 to 1 in crude sewage, 40 to 1 in the septic tanks and filter beds, and 70 to 1 in the sand filter effluents. Table 204 illustrates the numerical changes occurring during biological purification.

TABLE 204

BOSTON SEWAGE. (Modified from Winslow 1905.)

	Microscopic Count.	Gelatin.	Agar.	Microscopic ÷ Gelatin.	Agar × 100 ÷ Gelatin.
Crude sewage . .	29,000,000	1,690,000	1,400,000	17	83
Septic tanks . .	30,000,000	787,000	504,000	38	64
Contact filters .	24,000,000	521,000	432,000	46	83
Trickling filters .	17,000,000	451,000	284,000	39	63
Sand filters . .	65,000	9,160	10,800	7	120

It will be seen that no marked fall occurs in the number of living organisms till the sand filter stage is reached.

A method of sewage disposal that is now widely used is the Activated Sludge Process. Briefly this consists in treating the sewage with about 15 per cent. of bacterially active liquid sludge, in the presence of an ample supply of atmospheric oxygen. As a result of this treatment, a large proportion of the colloidal material undergoes coagulation, and subsequent sedimentation. The activated sludge itself results from the aeration of successive portions of sewage (see Martin 1927, Buswell 1928). As Wooldridge and Standfast (1936) have shown, the purification is brought about by a series of catalysed oxidation-reduction reactions determined by bacterial enzymes present in the living or dead bacterial cells or liberated by them into the fluid of the reacting system. A reduction of as much as 98 per cent. in the number of living bacteria may occur during the process (Miller 1936).

The survival of typhoid bacilli in sewage has been studied by a number of workers (see Green and Beard 1938). The rate at which they die out depends on a variety of factors. As a rule, the great majority are dead within a week and all within a month. Their death is probably accelerated by the activated sludge process of treatment, though observations on *Salm. typhi-murium* have shown that this organism may survive in drying sewage sludge for several months (Stokes *et al.* 1945).

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CHAPTER 93

THE BACTERIOLOGY OF MILK

Sources of Bacteria in Milk

EVEN when drawn with aseptic precautions, milk always contains a certain number of bacteria which are derived from the milk ducts of the **cow's udder.** Their numbers vary considerably from quarter to quarter and from cow to cow, and are highest in the fore-milk and lowest in the strippings. The average plate count of aseptically drawn milk has been given by different workers as less than 10 per ml. to several thousand. Many of the lower counts have probably underestimated the true number owing to unsuitable technique in counting, while many of the higher counts have probably been made on milk derived from udders that were not strictly normal. Von Darányi (1941), who took special care in preventing contamination of the udder and who discarded the fore-milk, found that nearly all healthy cows from the Veterinary High School herd yielded milk having a plate count less than 100 per ml. Strynadka and Thornton (1937), on the other hand, who used the direct microscopic method, found that the bacterial counts on 22 samples of aseptically drawn milk ranged from 10,000 to 370,000 per ml. Considering the extraordinary frequency of mastitis (see Minett 1932, Hucker *et al.* 1932, Diernhofer 1933), 50 per cent. of the animals in a herd often showing disease of one or more quarters, it is very difficult to arrive at any definite conclusion on the probable numbers of bacteria in milk coming from apparently healthy udders. If the udder is diseased, streptococci, *Br. abortus*, tubercle bacilli, and other organisms may be excreted in the milk in large numbers—sometimes running into several thousands per ml.

When aseptic precautions are not employed, the milk is liable to be contaminated with bacteria from the outside of the udder, the interior of milk vessels and utensils, and dust in the atmosphere of the milking shed. Of these various sources, far and away the most important is **unsterilized milking equipment.** The total number of organisms gaining access from the air and from dust is almost negligible compared with that derived from the surfaces of unsterilized pails, coolers, cans, strainers, clarifiers, and bottle fillers. Unless these utensils are actually sterilized—preferably by steam—their surfaces become coated with bacteria of various types, which may contribute enormous numbers of micro-organisms to the milk.

An additional source of contamination is the **human personnel.** If wet milking is practised, considerable numbers of organisms are liable to get washed into the milk from the milker's hands, as well of course as from the exterior of the udder and teats, unless these are thoroughly cleansed. Though numerically few, the organisms reaching the milk in this way may be of considerable public health importance, particularly if the milker happens to be an intestinal carrier of typhoid, paratyphoid, dysentery, or food-poisoning bacilli. The milker constitutes a further source of

danger if he is carrying hæmolytic streptococci or diphtheria bacilli in his throat or nose, since these organisms may gain access to the milk *via* the cough spray.

Apart from initial contamination of the milk, **imperfect cooling** is often responsible for the presence of large numbers of bacteria in any given sample. However carefully milk is produced, sooner or later, provided it is kept at a suitable temperature, it will go sour or putrid as the result of bacterial multiplication. If it is to remain sweet for more than a few hours, all milk should be cooled immediately after production to a temperature of 10° C. or below.

It may be noted that the **keeping quality** of the milk is determined partly by the degree of initial contamination and partly by the temperature at which it is kept. Milk produced under really cleanly conditions has a considerable bacteriostatic power, and shows little bacterial multiplication for several hours, even when incubated at a favourable temperature. On the other hand, the bacteriostatic effect of milk produced under dirty conditions is very slight, and bacterial multiplication sets in rapidly. For this reason it is much easier to distinguish between a milk produced under sanitary, and one produced under insanitary, conditions if the examination is delayed till the milks have stood at a temperature of 60° F. or so for 12–18 hours. After this time a milk produced under clean conditions will still have a low bacterial count, while a milk produced under dirty conditions will contain a large bacterial population.

The **production of clean milk** is largely a matter of technique, not of structural equipment or refinement. Provided all utensils are sterilized by steam, and the surface of the udder and the milker's hands are cleansed, it is possible to produce milk with a very low bacterial content even under unfavourable conditions. It is, however, not easy to maintain a satisfactory technique day in and day out unless suitable conditions and appliances are provided for the workers.

Types of Bacteria in Milk

The bacteria present in milk from healthy cows may be classified into the following groups:

1. Acid-forming Bacteria.—These organisms ferment the lactose in the milk with the production of acids, mainly lactic acid, which combine with the calcium caseinogenate, liberating free caseinogen; this, being insoluble in water, is precipitated in the form of a smooth, gelatinous curd. which shows little tendency to contract and squeeze out fluid. Lactic streptococci constitute some of the most important members of this group. One particular member is often referred to as *Str. lactis*, but as we have seen in Chapter 24 the definition of this species is very difficult Most of these organisms appear to be of vegetable origin, being found on corn, beans, peas, cabbage, etc. (Stark and Sherman 1935). Certain staphylococci, which are not infrequently found in the udder, are also active members of this group. We must further include the lactobacilli, which, however, under ordinary conditions do not appear to proliferate very actively.

2. Gas-forming Bacteria.—These organisms ferment lactose with the production of both acid and gas. They produce a smooth, gelatinous curd, which is often riddled with gas bubbles. The acid produced is largely acetic, which imparts an unpleasant flavour to the milk. Coliform bacilli are among the commonest representatives of this group, which includes, however, certain anaerobic organisms such as *Cl. welchii* and *Cl. butyricum*.

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3. Proteolytic Bacteria.—These organisms secrete one or other, or both, of two ferments—rennet and casease. Rennet acts in two stages; in the first stage the calcium caseinogenate is converted into a soluble form of casein; in the second stage this is precipitated as calcium caseinate by the calcium salts present in the milk. The resulting clot gradually contracts, and squeezes out a more or less clear fluid, which is known as whey; the whey contains the sugar, salts, and other proteins—lactalbumin and lactoglobulin—in the milk. Casease is a proteolytic enzyme, which digests the proteins present in the milk, breaking them down to proteoses, peptones, and amino-acids.

When the rennet predominates, a firm white curd is produced, which is slowly digested by the casease. When the casease is in excess, either no coagulation at all may occur, or a soft flocculent clot is formed, which is rapidly peptonized.

Many organisms fall under this heading: (*a*) spore-bearing aerobes, such as *B. subtilis* and *B. mesentericus*; (*b*) *Proteus vulgaris*; and (*c*) staphylococci and micrococci. Some of the organisms in this group also ferment lactose, so that the milk is rendered acid, besides being clotted and digested.

4. Alkali-forming Bacteria.—These organisms render the milk alkaline, presumably by acting on the proteins. Some of them also secrete lipase, and the combined effect is to saponify the fat, and convert the milk into a yellow translucent whey-like fluid. This action is not often seen, since the members of this group of bacteria are overgrown by members of other groups, which produce acid, coagulation, or peptonization. Organisms belonging to this group comprise *Bact. alkaligenes*, and some of the aerobic spore-bearers.

5. Inert Bacteria.—Many bacteria produce no visible change in milk and are hence called inert. Some of the udder cocci are included in this group. Members of the *Achromobacterium* group, most of which are probably derived from water, are also relatively inert. It may be noted that most of the pathogenic organisms which may be present in milk belong to this group.

Pathogenic Bacteria in Milk.—Apart from the organisms that we have described as being normally present in milk, there are others which are sometimes found in pathological conditions of the cow's udder, or which gain access to the milk from an infected water supply or from some person handling the milk.

Tubercle bacilli.—The presence of these organisms in milk depends on the frequency of bovine tuberculosis. In Great Britain this disease is very prevalent, about 40 per cent. of cattle reacting to the tuberculin test. Though it is true that tubercle bacilli may be excreted in the milk in the absence of any definite disease of the udder (Report 1909), some degree of tuberculous mastitis can generally be demonstrated—though not always clinically—in cows passing infected milk. It is probable that on an average about 0·5 per cent. of all milch cows in this country are excreting tubercle bacilli in the milk (see Chapter 59). The proportion of market samples of raw milk containing these organisms depends to a considerable extent on the degree to which the milk is bulked, but an average figure of about 7 per cent. may be taken for the country as a whole (Report 1932). The numbers of tubercle bacilli excreted by a single cow are variable from animal to animal, and from day to day, but it is probable that they may reach several hundred or even thousand per ml. (see Pullinger 1934).

Br. abortus.—This organism is responsible for contagious abortion of cattle, which is probably economically more important in this country than tuberculosis.

The udder is frequently infected, even in animals that have not aborted (see Chapter 75), though no lesions can be detected by clinical examination. The bacilli are excreted regularly or intermittently in the milk. Their numbers are usually greatest at the beginning of lactation, when they may reach as many as 200,000 per ml., but in the later stages they diminish and rarely exceed 2,000 per ml. (Stockmayer 1936). The udder may remain infected for years. About 30 per cent. of samples of raw mixed milk offered for human consumption appear to contain this organism.

Streptococci.—Mastitis streptococci are of various types, the most important being *Str. agalactiæ* (see Chapter 24). They are excreted in variable numbers in the milk of cows suffering from mastitis. This disease is extremely common, and affects not only the poorer herds, but even herds producing high-grade milk. Pullinger (1935), for example, found that the milk from 10 out of 12 herds producing Certified milk and 12 out of 14 herds producing Grade A tuberculin-tested milk contained mastitis streptococci when examined at intervals over a period of 15 months. The majority of streptococci causing mastitis are probably non-pathogenic for man, but occasionally a strain of human type invades the udder, and may be responsible for outbreaks of scarlet fever or septic sore throat in persons consuming the milk (see Bendixen and Minett 1938, Dublin *et al.* 1943). The milk may also be contaminated directly with these organisms from persons who are either suffering from streptococcal throat lesions, or who are carrying these organisms in their throat or nasopharynx (see Henningsen and Ernst 1939). Most outbreaks of milk-borne streptococcal infection appear to result from the former method of infection. Though pathogenic streptococci must often gain access to milk from human cases and carriers, their rate of multiplication in raw milk at ordinary temperatures is usually too slow for them to render the milk sufficiently infective to cause disease (see Pullinger and Kemp 1937).

Staph. aureus.—This organism is a not infrequent cause of mastitis. It is often found in the apparently healthy udder, but in cows suffering from mastitis it may be present in large numbers in the milk. Its public health importance lies in the fact that, under favourable conditions, it may multiply in the milk and give rise to a toxin capable of producing gastro-enteritis in human beings (see p. 1605).

C. diphtheriæ.—This organism occasionally finds its way into the milk from the throat or nasopharynx of a human carrier or case of diphtheria. Very occasionally it becomes implanted on ulcers on the cow's teats. Such an occurrence is peculiarly dangerous, since the milk is uniformly infected (for references, see Goldie and Maddock 1943).

Typhoid, paratyphoid, food-poisoning, and dysentery bacilli.—These important pathogenic organisms, which may occasionally contaminate the milk, are usually derived from human or other extraneous sources (see p. 1542).

Quality in Milk. Cleanliness and Safety

Quality is a composite, not a single, attribute of milk. There are, for example, (1) the nutritive quality, (2) the cleanliness, (3) the keeping quality, (4) the pasteurizability, and (5) the safety of milk, to mention only five of its most important attributes. Leaving aside the nutritive quality, which can be determined only by chemical analysis and animal feeding experiments, we may consider for a moment what we mean by the other four properties.

By **cleanliness** is generally understood the freedom of the milk from extraneous matter, from blood, and from an undue number of leucocytes and bacteria. It is an unsatisfactory term, but by general usage it has come to bear this connotation.

The **keeping quality** of the milk refers, of course, to the length of time it will remain sweet, and free from odours and tastes that render it unpalatable.

The **pasteurizability** of the milk is a term devised to indicate the suitability of the milk for heat treatment. It is, of course, a mistaken idea that any milk can be pasteurized. A milk that is too acid will clot when the temperature is raised, and the resultant product will have to be discarded. Again, as Anderson and Meanwell (1933) have shown, some milks contain large numbers of heat-resistant bacteria, which are not destroyed by pasteurization, and which therefore prevent the pasteurized product from conforming to the legal standard.

The **safety** of milk is a term denoting its freedom from bacteria capable of giving rise to disease in man or animals. It is by some writers confused with cleanliness, but there is no necessary or constant relationship between these two properties. Dirty milks, if free from pathogenic bacteria, may be quite safe, while very clean milks are not infrequently dangerous.

It has already been pointed out that pathogenic bacteria in milk may come from (1) the udder, such as the tubercle bacillus, *Br. abortus*, and some streptococci and staphylococci; (2) the infected nasopharynx of human beings handling the milk, such as hæmolytic streptococci and diphtheria bacilli; (3) excretal material gaining access either from the hands of human beings, or indirectly from water contaminated with urine or fæces—usually of human origin; in one or other of these ways typhoid, paratyphoid, dysentery, and food-poisoning bacilli may be carried into the milk. These are the organisms responsible for *dangerous* milk. Those responsible for *dirty* milk, on the other hand, come from various sources, particularly unsterilized milk utensils, caked mud and manure on the cow's udder, dirt on the milker's hands, and from the fore-milk in the teat canal. Provided the cow is healthy, provided that none of the human beings handling the milk is a carrier of pathogenic bacteria, and provided that the water supply is pure, dirt and bacteria may gain access to the milk in considerable quantities without endangering the health of those consuming it. On the other hand, if pathogenic bacteria find their way into the milk from one of the sources quoted above, no matter in how cleanly a manner the milk is produced, it is a potentially dangerous and unsafe milk.

These conclusions are borne out by epidemiological experience, which has shown that several outbreaks of disease have followed the consumption of milk of the highest standard of cleanliness. Without discussing this subject further, we shall probably be wise to regard cleanliness and safety as two entirely separate attributes of milk.

For human consumption it is desirable, of course, that milk should be both clean and safe. Clean milk is more æsthetically desirable, it has a better flavour, and it keeps longer. Moreover, it is not likely to contain any of those toxic substances resulting from undue bacterial proliferation, which have an irritating effect on the gastro-intestinal tract—particularly of infants (see Park and Holt 1903 and Chapter 72). The fewer organisms there are in milk, and the more bacterial proliferation is checked, the less liable is the milk to give rise to digestive disturbance of this type.

As regards safety, it will be clear that *no raw milk can ever be regarded as com-*

pletely safe for human consumption. The frequency of disease in cattle, the risk of contamination from human and other sources, and the suitability of the milk itself as a medium for bacterial multiplication, combine to render the consumption of raw milk potentially dangerous. The only satisfactory way of eliminating this danger is by pasteurization or some other form of heat treatment.

Bacteriological Grading of Milk

From a public health point of view it is usual to grade milk bacteriologically partly according to cleanliness and partly according to safety. We may consider briefly the various methods available for these purposes.

Grading according to Cleanliness.

For a detailed description of the methods used and a study of their experimental errors, and for a critical discussion on the interpretation of their results, the reader is referred to the monograph by Wilson and his colleagues (1935).

Sampling.—Since the answer given by any test is determined so largely (*a*) by multiple factors involved in the production and subsequent handling of the milk; (*b*) by the care with which the milk is sampled, and (*c*) by the time-temperature conditions under which the sample is taken and kept prior to analysis, it follows that it is unwise to pay too much attention to the results of any single examination. The most satisfactory procedure is to make frequent and regular examinations of the milk of any given producer throughout the year, and insist that a given proportion, for example 75 per cent., of the samples should come up to a given standard. In this way some allowance will be made for factors over which the producer is unable to exercise complete control, while at the same time ensuring that the conditions of production as a whole are kept at a reasonably high level.

Every endeavour should be made in the sampling of the milk to obtain a homogeneous distribution of organisms and fat. With regard to the subsequent treatment of the sample, two alternative procedures are available. If the intention is to test the cleanliness of production, it is desirable to expose the sample after collection to an agreed temperature for a given length of time, in order that the latent contamination in milk produced under bad conditions may have time to develop. When testing milk delivered to the consumer, on the other hand, it is desirable to ice the sample at once, so as to obtain information on the degree of bacterial contamination at the time of delivery by the distributor.

The sample may be examined by the following tests:

(1) **The Sediment Test.**—The amount of extraneous matter in the milk is determined either by filtration through a cotton pad, or by centrifugation. A simple sediment test of this sort is of value in controlling the gross dirt in milk and in education of the unhygienic farmer. Since, however, filtration through muslin gauze on the farm will remove dirt of this type, the absence of an obvious sediment must not be taken to mean that the conditions of production were satisfactory. Most of the bacteria in milk come, not from gross dirt, but from unsterilized utensils. Indeed, actual manure can be added to the milk without producing any marked increase in the bacterial content; for this reason a sediment test is useful for the detection of gross particulate matter, the presence of which is not likely to be detected by purely bacteriological tests.

(2) **The Cellular Content.**—Normal milk contains various types of cells, the number of which is estimated by different workers at between about 200,000 and 1,000,000 per ml. These estimates probably err on the high side, though it may be noted that Strynadka and Thornton (1937) on a series of seven milks obtained cell counts ranging from 80,000 to 5,100,000 per ml. According to Prouty (1934), milk from perfectly normal udders contains, as a rule, less than 100,000 cells per ml. But the perfectly normal udder is almost a curiosity, and for practical purposes, therefore, we must allow a considerable margin on this figure. An abnormally high cell count may be due to retention of the milk in the udder, which leads to desquamation of the epithelial cells in the alveoli and ducts, to acute or chronic inflammation, which brings about a rise in the polymorphonuclear leucocytes, or to some other cause. A mere count of the cells present affords little information about the quality of the milk, and it would be unwise to condemn a milk solely on account of an increased cell content. If long-chained streptococci are found in addition, the probability is that the cow is suffering from mastitis. Much more information can be obtained by a differential count, but the real value of this method lies in the help it gives to the veterinarian who is engaged in a study of the individual cow.

(3) **The Breed Smear Method.**—The general technique of this method is to spread 0·01 ml. of the milk over 1 sq. cm. on a glass slide, to fix and de-fat, to stain with methylene blue, to count the number of individual organisms—the Breed "individual" count—or preferably the number of groups of organisms, individual organisms each being regarded as a group—the Breed "clump" count—in a given number of fields, and finally to calculate the number of organisms in 1 ml. of the original milk. The result obtained affords an index of the total *stainable* organisms present, not necessarily of the total organisms. The chief value of this method lies in its rapidity, which enables an opinion to be formed on the bacterial cleanliness of a milk within a few minutes. It is particularly suitable for use in collecting stations to which milk from individual farms is brought before being mixed and sent up to towns in large rail or road tanks. Unsatisfactory milks, which would taint the rest, can be picked out quickly, and returned to the producer. With a little experience, a mere glance over a few fields is sufficient to indicate the general quality of the milk. The Breed Smear method is also of value to the farm inspector in indicating the probable nature of the defect in unsatisfactory milk. Thus the presence of large masses and clumps of bacteria suggests unsterilized milk utensils; the presence of numerous organisms arranged in pairs or small units suggests inadequate cooling; the presence of long-chained streptococci associated with a high leucocyte count indicates mastitis, and so on (see Breed 1929).

(4) **The Keeping Quality Test.**—The milk is kept in a special bottle at a given temperature, such as 60° F. or 70° F., and examined every 8 or 12 hours until it becomes sour or putrid. As a general method for controlling the hygienic quality of the milk, this test performs a useful function, but the fact that the end-point is entirely subjective necessarily exposes the result to a very large experimental error. Some degree of objectivity can be introduced into the test by the use of alcohol. If 1 ml. of a 68 per cent. solution of ethyl alcohol is added to 1 ml. of the milk and the tube is inverted, precipitation of the casein occurs in milks that are near the souring point. On the whole, there is good agreement between the taste and the alcohol tests, the latter being slightly more sensitive (see Anderson and Wilson 1945). An indirect method, which appears to afford a good indication

of the keeping quality, is a modified methylene blue test carried out at 60° F. (see Provan, Dudley and Thomas 1936).

(5) **The Plate Count.**—This is one of the most widely used methods for the bacteriological grading of milk according to cleanliness. It is, however, exposed to criticism on so many grounds that it is doubtful whether its continued use is justifiable. Ostensibly the plate count measures the numbers of bacteria in the milk, but in fact it does not. The bacteria in raw milk are of many different kinds and come from varied sources. Often a large proportion are dead, while those that are alive differ in their nutritional, respiratory, and temperature requirements, so that no one medium incubated for a given length of time at a given temperature under aerobic conditions can possibly afford a true estimate even of the living organisms present. More important still, however, is the fact that many of the bacteria are distributed, not individually, but in chains and groups of varying sizes. The colonies developing on plates are derived therefore, not solely from individual organisms, but also from aggregates containing varying numbers of bacteria. In consequence the plate count merely registers the number of bacterial particles capable of forming colonies under the particular conditions selected. Since the average number of bacteria per clump is variable from one milk to another, and not constant from time to time even in milk of the same origin, it follows that the figures yielded by the plate count are arbitrary, are not strictly comparable, and have no absolute significance.

When it is further pointed out that these clumps of bacteria may disintegrate to a variable and quite uncontrollable extent during the process of dilution, leading in extreme instances to errors of 1,000 per cent. (Ward 1926), and that many of the individual steps in the technique of the count are very difficult to standardize, and even when standardized as nearly as possible are attended by a large experimental error, it will be realized that the figures yielded by the plate count are not merely arbitrary, but are also only approximate. (For a description and numerical assessment of the various errors of dilution, incubation, counting, personal bias and personal variability, sampling, overcrowding, and so on, see Wilson *et al.* 1935.)

Even were it possible to standardize the technique of the plate count, it would still be necessary to allow a margin of $\pm$ 50–90 per cent. on the result of any one milk, depending on the number of plates used for each dilution. Even this estimate would often fall short of the real variation met with in practice (Mattick *et al.* 1935). However accurate the plate count may be for the enumeration of bacteria in pure broth cultures, it is plainly unsuitable for this purpose when applied to milk. Because the results are expressed quantitatively in figures extending over a wide range, they afford a fictitious appearance of accuracy which leads, not only in laymen, but even in public health officials, to a wholly unjustifiable feeling of confidence in their value. It is not denied that the plate count can be used as a rough yard-stick for the grading of milk, but it is denied that it can be used as a millimetre rule. As scientific bacteriologists we can hardly condone a practice which is little short of bacteriological prostitution.

(6) **The Coliform Test.**—Like the plate count, this method has been extensively used in the past for the grading of milk without any real appreciation of the fundamental assumptions on which it rests. We have seen that in the analysis of *water* the coliform test affords us the most delicate index we have of excretal pollution. The main reasons for this are that (1) in this country something like 90 per cent. of waters giving a positive presumptive coliform reaction in MacConkey broth are

proved on further examination to contain typical *Bact. coli* of excretal origin (Bardsley 1934); (2) organisms of the *Bact. coli* type in water in this country are very frequently derived from human excretal material, and since human fæces and urine may contain pathogenic bacteria, the presence of any excretal pollution, as indicated by the coliform test, must be regarded as potentially dangerous; and (3) as a rule, coliform organisms do not seem to multiply in water under natural conditions; on the contrary, they tend to die out rather rapidly (Houston 1913). In this country, therefore, the coliform count in water indicates the minimal amount of excretal pollution that has occurred.

When we turn to *milk* we find that (1) a considerable proportion—something like 50–70 per cent.—of raw milks in this country in which coliform bacilli are found contain not the true *Bact. coli* but organisms of the intermediate or aerogenes-cloacæ types. These organisms come mainly, not from fæces and urine, but from soil and grain, and their presence therefore in milk cannot be regarded as an index of excretal pollution; (2) the true *Bact. coli* organisms that are found in milk appear to come either directly from cow-dung and manure, or indirectly from unsterilized milk utensils in which bacterial multiplication has occurred. If they are derived from the latter source, they clearly afford no index of *direct* excretal pollution. If they are derived from the first source, their presence may be considered objectionable on the ground that organisms pathogenic for man are sometimes present in the intestinal canal of the cow. The presence, however, in milk of excretal material of bovine origin must be regarded as very much less dangerous for man than that of human origin. (3) Unlike water, milk affords an admirable medium for the growth of coliform bacilli. If it is kept at a temperature of 50° F. or over, a great increase in their numbers may take place, a rise of several thousand-fold often occurring at 60° F. within 24 hours (see Ayers and Clemmer 1918, Finkelstein 1919, Sherman and Wing 1933). Unless, therefore, the milk has been kept at 40° F. or less, an estimation of the coliform bacilli affords only a very imperfect, and often entirely misleading, index of the extent of the original contamination. With a milk produced and kept under ordinary conditions, it is impossible to tell how many of the coliform bacilli gained access at the time of production, and how many are due to the multiplication of those originally present.

It will thus be seen that none of the three premises on which the scientific application of the coliform test to water in this country is based holds true for milk. Even a differentiation of the coliform group into its constituent types is not likely to help us, since (*a*) it is impossible to tell how many of the different types were original contaminants and how many have resulted from growth, and (*b*) organisms of the fæcal coli group are not necessarily derived directly from excretal material, but may come from unsterilized milk utensils on which they have been multiplying perhaps for several generations.

The presence of coliform bacilli in small numbers in water or milk is not in itself objectionable; it is merely as an index of the possible accompanying presence of pathogenic organisms that it is of importance. In milk, the coliform test cannot be used as an index of direct excretal pollution, for though it is true that if manure gains access to milk, coliform organisms will probably be found, the reverse conclusion does not hold true. There are so many other sources on the farm for these organisms that their presence in milk cannot be held to justify the conclusion that the milk has necessarily been contaminated with excretal material. The coliform test on milk fails to provide us with that specific qualitative information which it

supplies in the case of water, and as a general method of grading, therefore, it seems to be unsuitable. It may serve a useful purpose in the examination of the highest quality raw milk, since such milk often fails to show the presence of coliform bacilli in 1 ml. quantities even several hours after production (Mattick and Williams 1925). Its use, however, in this connection is purely empirical, merely serving as an index of contamination of the milk from environmental sources. Again the test may be of some empirical value in the control of pasteurization, since the practical absence of coliform bacilli from pasteurized milk examined immediately after processing affords, except with very clean milks, a fairly good indication that pasteurization has been carried out efficiently.

(7) **The Modified Methylene Blue Reduction Test.**—The general technique of this test is to add 1 ml. of a standard methylene blue solution to 10 ml. of milk in a test tube, to insert a sterile rubber cork, to invert the tube once or twice so as to mix the dye with the milk, to incubate in a constant-temperature water-bath at 37°–38° C. in complete darkness, to invert the tube every half-hour, and to observe the time at which the dye is decolorized. If reduction has not occurred within a given time, then no further observations need be made (see Wilson *et al.* 1935).

Reduction depends partly on the absorption of oxygen and the production of reducing substances by the organisms, and partly on the presence of a natural reducing system in the milk itself (see Hobbs 1939). Since some of the reducing enzymes and some of the organisms are adsorbed on to the fat, and since the fat globules themselves play an important mechanical part in increasing the surface available for enzyme action and in affecting the visual depth of colour, the necessity of preventing an accumulation of cream at the surface will be realized; hence the necessity of half-hourly inversion.

The rate at which methylene blue is reduced in milk depends partly on the number of organisms and partly on their metabolic activity. For this reason it affords a very good index, not only of the bacterial cleanliness of the milk, but also of its keeping quality. The test is unsuited for the milk of individual cows, and for freshly pasteurized milk, but affords quite a good index to the keeping quality of pasteurized milk as delivered to the consumer (Ciani 1939). Its chief value lies in affording a fairly rapid means of assessing the general hygienic quality of raw mixed milk. Its technical simplicity, its cheapness, and its extremely small experimental error render it admirably suited for the routine control of this type of milk. The reduction time to be laid down must depend on the level of cleanliness desired. A milk of high quality, which has been kept for about 12 hours at atmospheric temperature in this country, should not reduce methylene blue in less than about 8 hours in the winter or 7 hours in the summer, while one of fairly good quality should not reduce the dye in less than about 6 and 5 hours respectively.

Besides the advantages already noted, the reduction test has certain other advantages over the plate count. The first is that the result does not appear to be affected to any considerable extent by the degree of aggregation of the bacteria, which is one of the most disturbing features of the plate count on milk. The second is that the test affords a much more delicate index of bacterial growth than the plate count. It will be remembered (Chapter 4) that during the lag phase bacteria increase in size for some time before they commence to divide. The plate count therefore remains approximately constant during this phase. The reduction time, on the other hand, falls rapidly, owing to the active metabolism of the organ-

isms. It serves, in fact, as a remarkably sensitive index of growth, and is hence peculiarly fitted to gauge the keeping quality of the milk. The plate count yields a *static* picture of the bacterial population: the methylene blue reduction test affords a *dynamic* picture. (For comparison between the two tests see Wilson *et al.* 1935, Nichols and Edwards 1936, Asdrubali 1939, Barkworth, Irwin and Mattick 1941).

(8) **The Resazurin Test.**—The principle of this test, which owes its practical introduction mainly to the work of Ramsdell, Johnson and Evans (1935), is the same as that of the methylene blue test. The test consists essentially in adding to the milk a solution of resazurin to give a final concentration of 1/200,000, incubating at a given temperature, and observing the time taken to bring about a change in the colour of the dye, or alternatively noting the colour reached after a given time. The colour passes from blue, through various shades of purple and mauve, to pink, and ultimately to complete decolorization. The change from blue to pink is due to the irreversible reduction of resazurin to resorufin: the change from pink to colourless is due to the reversible reduction of resorufin to hydroresorufin. The colour can be assessed by a suitable comparator. Numerous methods of performing this test have been described (see Johns and Howson 1940, Davis, Newland and Knuckey 1943). In this country a 10-minute test is used for the control of "platform" milk, that is producers' milk arriving at a creamery, in order to see whether it is clean enough to be bulked with other milk. A longer test is employed by agricultural bacteriologists for the control of the general quality of milk at the time of production.

The advantages of the resazurin over the methylene blue test are mainly two. Firstly, resazurin changes in colour rather more rapidly, and though complete reduction takes as long as or even longer than methylene blue, useful information can be obtained by noting a change in colour from blue to lilac or pink. Secondly, resazurin is more sensitive to the cell content of milk, and is therefore regarded by some workers as of value in the detection of mastitis. The practical importance of these two advantages is dubious. Admittedly a 10-minute test is useful as a screen for grossly contaminated milk; but if the resazurin test is to be used as an index of keeping quality and the reduction of the dye observed to the pink stage, then there is practically no saving of time over the methylene blue test (Anderson and Wilson 1945). Watts and Stirling (1944), in a very careful investigation, found that the resazurin test, even when carried out on individual quarter samples, was of limited value for the routine diagnosis of mastitis, and practically valueless for the detection of milk from infected cows when mixed with normal milk; in fact, as much as 73 per cent. of the herd milk could be derived from cows suffering from mastitis without significantly altering the result of the resazurin test.

The disadvantages of the resazurin test are that the dye is not easy to standardize and is very sensitive to sunlight, that the colour change has to be followed by a comparator, and that the lilac and pink stages are not easily appreciated by persons whose vision is insensitive to red colours. In general terms it may be said that the resazurin test is more influenced by abnormal milk such as colostrum, late-lactation milk, milk from cows with mastitis, and by milk containing organisms with low reducing powers, but that the methylene blue test affords the better indication of bacterial contamination of the milk.

(9) **The Laboratory Pasteurization Test.**—This consists in making a plate count

before and after pasteurization in the laboratory, and noting the numerical reduction that has occurred (Anderson and Meanwell 1933). Its main purpose is to detect the presence of heat-resistant and thermophilic bacteria in the milk, which may be present in such numbers as to prevent the pasteurized product from conforming to the official standards laid down. It is essentially a test for use in processing depots.

It will be realized that numerous tests are available for assessing the general cleanliness of milk, and that each of these tests affords an answer to a different question. Complete correspondence of the results of the different tests cannot therefore be expected. Experience has shown, however, that the Breed clump count, the plate count, and the modified methylene blue reduction test are all fairly highly correlated with each other. On individual milks, of course, the reduction test and the plate count may give widely different results, owing mainly to the extraordinary high experimental error of the plate count, but on an average the results of these tests are very similar. For practical purposes, therefore, there seems to be no advantage in using the complex, expensive, and highly inaccurate plate count in preference to the simple, inexpensive, and accurate reduction test. From a public health point of view, probably only two divisions need be made on the basis of *cleanliness*, namely (*a*) into milk that is suitable and (*b*) into milk that is not suitable for human consumption in the liquid state. We would again emphasize that a *clean* milk is not necessarily a *safe* milk.

Grading according to Safety.

The pathogenic organisms for which search is commonly made in milk are the tubercle bacillus, *Br. abortus*, and hæmolytic streptococci. The methods employed in searching for the first two organisms have already been described in Chapters 59 and 75 respectively.

The presence of mastitis streptococci in milk may be demonstrated microscopically or by culture, but according to Minett (1935) the microscopic method detects only about half of the cases that are positive culturally. The cultural method is, therefore, the method of choice. Individual quarter samples of the fore-milk should be plated out on to a suitable blood agar medium and a study made of the colonies that develop (see p. 565). The differentiation of *Str. agalactiæ* from other closely related types has been discussed in Chapter 24.

Milk Designations.—The grades of milk in England and Wales are defined in the Milk (Special Designations) Orders and Regulations 1936 to 1944. Briefly, four main grades of milk are laid down—Tuberculin Tested, Accredited, Pasteurized, and Heat-Treated. Some of the more important requirements affecting the production and testing of these milks are set out in Table 205.

It will be noted that (1) Tuberculin Tested milk may be pasteurized: (2) Accredited milk does not have to come from herds subjected to a tuberculin test, and hence cannot be guaranteed to be free from tubercle bacilli; (3) Milk may be pasteurized either by the Holder method at 145°–150° F. for 30 minutes or by the High Temperature Short Time method at 162° F. for 15 seconds; (4) No regulations are laid down for the production of Heat-Treated milk; (5) Tuberculin Tested (Pasteurized) and ordinary Pasteurized milk have to conform to the standard of Heat-Treated milk as well as to a plate count test; (6) There is no official designation for ordinary raw milk, and no bacteriological standard of cleanliness

TABLE 205

GRADES OF MILK LAID DOWN IN THE MILK (SPECIAL DESIGNATIONS) ORDERS AND REGULATIONS 1936–1944 FOR ENGLAND AND WALES.

Designation.	General Conditions.	Bacteriological Requirements.	Remarks.
Tuberculin tested.	Tuberculin test on herd at least every 6 months. Veterinary inspection every 6 months.	Must not decolorize methylene blue in 4½ hrs. 1st May to 31st Oct., or in 5½ hrs. 1st Nov. to 30th April.[1] Coliform bacilli absent from 1/100 ml.	(i) If bottled on farm, may be labelled Tuberculin Tested (Certified). (ii) If pasteurized, must not decolorize methylene blue in 30 mins.,[2] must not give a phosphatase reading of over 2·3 Lovibond units, and must have a plate count not exceeding 30,000 per ml.
Accredited.	Veterinary inspection every 3 months.	Same as for Tuberculin Tested milk.	If bottled on farm, may be labelled Accredited (Farm Bottled).
Pasteurized.	Milk must be kept at a temperature (i) of 145°–150° F. for not less than 30 mins. and be immediately cooled to 55° F. or below, or (ii) of not less than 162° F. for at least 15 seconds and be cooled as above.	Must not decolorize methylene blue in 30 minutes,[2] must not give a phosphatase reading of over 2·3 Lovibond units, and must have a plate count not exceeding 100,000 per ml.	Pasteurizing plant must be approved by the licensing authority, and must be equipped with indicating and recording thermometers. Temperature records must be preserved for at least a month. High Temperature Short Time plant must be provided with an automatic flow-diversion valve. The milk shall not be heated more than once.
Heat-treated.		Must not decolorize methylene blue in 30 minutes,[2] and must not give a phosphatase reading of over 2·3 Lovibond units.	No method of processing the milk is laid down. Any milk, therefore, including sterilized milk, that conforms to the bacteriological requirements may be described as Heat-Treated milk.

[1] If the sample is taken before the milk has been placed in bottles or other containers for delivery to the consumer (*i.e.*, producers' milk), it must be kept at atmospheric temperature till 6 p.m. if it is a morning milk, and until 10 a.m. on the following day if it is an evening milk, before it is tested bacteriologically. It is not necessary for every sample to be submitted to both reduction and coliform tests as a routine; it will generally be found convenient in testing producers' milk to have most of the samples examined by the methylene blue test alone, reserving the coliform test for occasional use (see Memo. 1937).

[2] The sample should be taken on the day of delivery, and must be kept at atmospheric shade temperature in transit to the laboratory. In the laboratory itself it must be kept at atmospheric shade temperature not exceeding 65° F. until between 9 a.m. and 10 a.m. on the following day, when the methylene blue test is begun. At no time after sampling should the milk be exposed to the direct rays of the sun (see Report 1944).

or safety is laid down for this type of milk. From the point of view of public health Tuberculin Tested (Pasteurized), Pasteurized and Heat-Treated are the only milks that can be relied upon as being free from the risk of causing disease. All other milks should be heated to the boiling-point before being consumed in the liquid state, if it is desired to ensure the destruction of pathogenic organisms.

Diseases borne by Milk.—From the description that has already been given of the pathogenic organisms that may be found in milk, it will be realized that milk serves as an important vehicle in the spread of disease to man. The frequency of tuberculosis of bovine origin and of undulant fever due to milk-borne organisms has been considered in Chapter 59 and 75 respectively. In these diseases infection of the milk occurs in the udder of the cow itself. In scarlet fever, septic sore throat, and diphtheria, infection occurs from the udder of the cow or from the nasopharynx of some person handling the milk, while in typhoid fever, paratyphoid fever, dysentery, and food poisoning the causative organisms generally gain access to the milk from the infected hands of some human attendant, or probably less frequently from a contaminated water supply.

The true frequency of milk-borne disease is extremely difficult to estimate, partly because the disease is often insidious in its onset and suspicion of the milk supply is not aroused, and partly because isolated cases of milk-borne infections, even if they are recognized as such, are not usually recorded (see Bendixen *et al.* 1937). In this country Savage (1912) attempted to collect the data referring to milk-borne epidemics prior to 1912. Those from 1912 to 1937 have been collected by Wilson (1942) and are summarized in Table 206.

TABLE 206

RECORDED OUTBREAKS OF MILK-BORNE DISEASE IN GREAT BRITAIN IN 1912–1937 (EXCLUDING TUBERCULOSIS AND UNDULANT FEVER, AND OUTBREAKS DUE TO MILK PRODUCTS SUCH AS ICE-CREAM, CHEESE, AND CREAM BUNS). (Wilson 1942.)

Disease.	No. of Outbreaks.	No. of Outbreaks in which Number of Persons affected is stated.	No. of Persons affected in these Outbreaks.
Scarlet fever and septic sore throat	40	34	5,331
Diphtheria	20	18	773
Typhoid fever, paratyphoid fever, and dysentery	39	36	3,229
Gastro-enteritis	14	12	4,346
Total	113	100	13,679

These outbreaks merely represent those of which records are readily available, and almost certainly underestimate the real number. They pay no attention, of course, to sporadic cases of milk-borne infection, nor do they include the 6,000 cases of tuberculosis of bovine origin nor the 400–500 cases of undulant fever, in a large proportion of which infection is milk-borne, that occur annually in Great Britain.

In the United States Armstrong and Parran (1927) record data referring to 612 milk-borne epidemics occurring during the preceding 20 years (Table 207).

TABLE 207

OUTBREAKS OF MILK-BORNE DISEASE IN THE UNITED STATES, 1906–26. (Armstrong and Parran 1927.)

Disease.	No. of Outbreaks.	No. of Cases (incomplete).	No. of Deaths (incomplete).
Typhoid fever	479	14,968	219
Paratyphoid fever	7	434	15
Diarrhœa and dysentery	6	92	5
Septic sore throat	42	21,045	139
Scarlet fever	40	3,939	20
Diphtheria	26	971	7
Miscellaneous diseases	12	878	5
Total	612	42,327	410

This table again affords merely a conservative estimate of the frequency of milk-borne epidemics, and there is reason to believe that many outbreaks are not recognized or are not recorded (Brooks 1933). It will be noted that the relative incidence of enteric fever is very much higher than in Great Britain. During the years 1938 and 1939 the number of outbreaks in the United States transmitted by milk and milk products was 42 and 41 respectively, affecting 1,685 and 2,509 persons (Frank 1940, Fuchs 1941).

Dolman (1941) in Canada collected records of 67 milk-borne outbreaks during the years 1912 to 1939 affecting 8,348 persons; 51 of the outbreaks were of enteric fever and resulted in no fewer than 729 deaths.

We have no space to discuss the epidemiology of milk-borne disease. Apart from the explosive nature of the outbreak, it is interesting to note that in milk-borne scarlet fever and diphtheria the average age incidence is often very much higher than in non-milk-borne epidemics. The majority of the cases may occur in persons of 15 years and over (see Godfrey 1929, Clarke 1936, Hill and Mitra 1936).

Methods for increasing the Safety of the Milk Supply.

These fall into three main categories.

A. Control of Animal Disease.—Reference has already been made in Chapters 59 and 75 to the methods available for diminishing the frequency of tuberculosis and contagious abortion in cattle, and in Chapter 67 to the elimination of mastitis. The success attendant on these measures will depend very largely on the vigour with which they are prosecuted. The eradication of disease, however, from the animal population is essentially an agricultural and veterinary problem to be undertaken in the economic interests of the farmer, and the task, particularly in this country where cattle disease is so widespread, is bound to be a long one. However much, therefore, we may support our veterinary colleagues in their enterprise, we must remember that this mode of approach can afford only a tardy and a partial solution to the problems faced by the public health administrator, namely, that of supplying a safe milk to the human population.

B. Control of the Human Personnel handling the Milk.—However healthy the animals producing the milk may be, there is always danger of its becoming infected directly or indirectly from the nasopharynx or fæces of human carriers of *Str. pyogenes*, *C. diphtheriæ*, *Salm. typhi*, *Salm. paratyphi A* and *B*, dysentery bacilli,

and bacilli of the food-poisoning group. In the United States some attempt has been made to control the human personnel handling the milk. The most frequent examinations made comprise a physical inspection, a careful inquiry into the history of infectious disease, a series of fæcal and urinary examinations for organisms of the enteric and dysentery groups, and nose and throat swabs for streptococci and diphtheria bacilli (see Borman *et al.* 1935). There is no question that close supervision of milk operatives, particularly of those working in pasteurizing depots, is highly desirable. The procedure is, however, costly and is not free from administrative difficulties. Moreover, since the carrier state is often transient or intermittent, it is impossible without almost daily supervision to detect all dangerous persons.

C. Heat Treatment of the Milk : Pasteurization, Boiling, and Sterilization.—The dangers of infection of milk from cattle, from the human personnel, and from water are so great that we shall not be overstating the case if we assert that no raw milk can be regarded as perfectly safe for human consumption. Clearly we must interpret this axiom with discretion, remembering always that the stringency of our precautions must be commensurate with the size of the population at risk. It is very difficult to assess in any particular instance the real danger run by persons consuming a given milk supply, and our safest course is therefore to insist as far as possible that all pathogenic organisms shall be destroyed by some form of heat treatment.

Pasteurization affords the most satisfactory means for this purpose. In this country two methods are permitted. The Holder process consists in raising the milk to a temperature of 145–150° F., holding it at this temperature for 30 minutes, and immediately cooling it to 55° F. or below. If this process is carried out in properly designed plant free from mechanical defects (see Scott and Wright 1935, Dalrymple-Champneys 1935), and supervised by intelligent and conscientious operatives, it can be relied upon to destroy all pathogenic organisms in the milk. In the so-called High Temperature Short Time process the milk must be kept at a temperature of not less than 162° F. for at least 15 seconds and then cooled to 55° F. or below.

The adequacy of heat treatment may be controlled by the *phosphatase test* (Kay and Graham 1935, Kay, Aschaffenburg and Neave 1939, Memo. 1943, Report 1944), which depends on the fact that the enzyme phosphatase, which is normally present in milk, is destroyed by a temperature of 145° F. within 30 minutes or of 162° F. within 15 seconds. The test is carried out by incubating the milk in the presence of a buffered solution of disodium phenyl phosphate. The amount of phenol that is liberated is measured by adding Folin and Ciocalteu's reagent and estimating the depth of the resulting blue colour by a Lovibond tintometer or, more simply and approximately, by a Lovibond comparator. Raw milk gives figures of about 50 blue units, but in properly pasteurized milk the number of units is reduced to 2·3 or less. This is a most valuable test and can be used in practice to ensure that the processing of the milk is carried out satisfactorily. Phosphatase is slightly more resistant to heat than the tubercle bacillus, so that a negative phosphatase result is strong presumptive evidence in favour of the safety of the milk.

The objections that have been raised to pasteurization, though often made on apparently scientific grounds, are determined almost entirely by economic or political motives. The farmer, especially the producer-retailer, may not obtain

such a high return for his milk if he has to pasteurize it himself or send it to a pasteurizing depot as if he sells it directly to the customer in its original raw and possibly dangerous state. Producer-retailers, moreover, are afraid that pasteurization will lead to their being gradually absorbed by the big milk combines, which can of course afford to pasteurize and bottle their milk under very much more favourable conditions than is possible for a small producer. Though appreciating the force of these motives, we must not allow the public health of the nation to be sacrificed to the financial interests of a small section of the community.

One of the chief objections that has been raised is that pasteurization lowers the nutritive value of the milk. The evidence for and against this contention has been reviewed by several workers (see Stirling and Blackwood 1933, Report 1934, 1936, 1937–39, Bendixen *et al.* 1937, Wilson 1942). The main effects of pasteurization by the Holder method at 145°–150° F. for half an hour followed by immediate cooling to 55° F. or below are as follows: (1) the cream line is reduced by about 10–30 per cent. (2) About 5 per cent. of the lactalbumin is coagulated. (3) There is a diminution of about 5 per cent. in the soluble calcium and phosphorus. (4) There is some destruction, amounting usually to about 10 per cent., and at the most to 25 per cent., of Vitamin B_1. (5) There is a variable diminution in the Vitamin C content, dependent on the degree of previous exposure of the milk to light, the presence of copper in the pasteurizing plant, and the amount of dissolved oxygen; at present, the average reduction is about 20 per cent. (6) Some of the iodine —almost certainly less than 20 per cent.—may be driven off as the result of volatilization. (7) A high proportion of the bacteria in the milk are destroyed, leading, as a rule, to a considerable increase in the keeping quality. Against these positive changes may be set a number of negative findings. Thus it is found from feeding experiments on rats and calves that the biological value and digestibility of the proteins is not decreased, that there is no detectable change in the availability of calcium or phosphorus, that there is no loss of Vitamin A or D, and that the total energy of the milk remains unaltered. So far as human beings are concerned, it may be pointed out that milk is a poor source of Vitamins C and D, and that infants fed on milk, whether raw or pasteurized, should therefore always be given a supplement of fruit or vegetable juice and cod-liver oil. The differences between raw and pasteurized milk are far less than those between cows' milk and human milk, or even between summer and winter samples of raw milk (see Kay 1939). Extensive feeding experiments on rats, mice and calves, and comparative investigations on children have failed to show that pasteurization has any significant effect in lowering the total nutritive value of the milk for the growing animal, and no fear need therefore be entertained that a policy of pasteurization will prejudice the nutritional development of the child population.

From a public health point of view there are manifestly strong reasons why all milk intended for consumption in the liquid state by a community of any size should be submitted to pasteurization, the process being, of course, adequately controlled by the appropriate supervising authorities. There is reason to believe that if pasteurization was rendered compulsory and universal, milk-borne disease would practically cease to exist (see Wilson 1942).

If pasteurization is impracticable, then the milk should be *boiled* in the individual household. The most satisfactory way of doing this is to bring it to the boil in a closed vessel, preferably a double saucepan, and cool it immediately to as low a temperature as possible.

Sterilization is the term used for a process which is applied commercially, and which is intended to destroy all but the most resistant spore-bearing organisms. In some plants the temperature is not raised above boiling-point, but more usually the milk is heated under a slight pressure. It is distributed in hermetically sealed bottles. Bacteriologically the milk is perfectly safe, but the partial caramelization which it undergoes renders its flavour unpalatable to most persons.

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INDEX

All organisms are listed under the generic names given in the Classificatory Chart on p. 319. Since many rod-shaped organisms have in the past been known under the generic name of *Bacillus*, irrespective of whether or not they belonged to this genus, we have for the convenience of the reader included most of them under the heading Bacillus (non-italicized). This heading should therefore be consulted by those who are doubtful to what genus a given organism belongs.

de Aar disease, 1648
Abel-Loewenberg bacillus, 494, 667, 1789
Abortin. *See* Brucellin
Abortion, contagious, in cattle, 1708
  diagnosis of, 1711
  epizootic, of mares, 1968
  human, due to *Br. abortus*, 1698
  infection of calves in, 1711, 1716
  infection of milk in, 1709, 1713
  infectious, in cattle and sheep due to *Vibrio fetus*, 1720
  mode of infection in, 1710
  prophylaxis of, 1715
    by eradication, 1715
    by vaccination, 1716
Abortus fever. *See* Undulant fever
Accessory food factors in bacterial growth. *See* Vitamins
Acetobacter aceti, 505
Acetyl-choline, relation to anaphylactic shock, 1150
Achalme's bacillus. *See Clostridium welchii*
Achromobacterium, 640, 641
Acid agglutination, 213, 692
Acid-fast bacilli. *See Mycobacterium*
Acid-fastness, 38
  cause of, 407
Acidophilic bacteria, definition of, 757. *See also Lactobacillus*
Acids, effect of on bacteria, 118, 2017. *See also under* pH
Aciduric bacteria, definition of, 757. *See also Lactobacillus*
Acne bacillus, 471, 1403, 1492, 1505, 1506
Acriflavine, disinfection by, 135
  for distinguishing smooth and rough organisms, 823
Actinobacillosis, 389, 1270, 1279–1281
  differentiation of, from actinomycosis, 1280
  pneumonia in calves due to *Actinobacillus actinoides*, 1281
Actinobacillus, **389–393**
  actinoides, 391, 1270
    causing pneumonia in calves, 1281
    in otitis media of rats, 1499
  actinomycetem-comitans, 391, 1274
  lignieresi, 389, 1270
    disease due to, 1279
    tabulated reactions of, 392
Actinomyces, **373–389**
  acid-fast types of, 375, 381, **387**
  aerial hyphæ of, 377
  antigenic structure of, 379
  biochemical characteristics of, 378
  classification of, 380
  cultural reactions of, 376
  culture clubs of, 375
  Drusen of, 375
  growth requirements of, 378
  habitat of, 374
  human and bovine types of, 379
  in mouth and salivary calculi, 1272
  morphology of, 374
  non-acid-fast types of, 375, 381, **387**
  pathogenicity of, 379
  pigment formation of, 379
  resistance of, 378
  spores of, 375
  tabular differentiation of, 392
  tissue clubs of, 375
  variation in, 379
  asteroides, 388, 1275
  bovis, 373 *et seq.*, **382**, 1270
  caprœ, 387, 1276
  farcinicus, 387, 1276
  graminis, 373 *et seq.*, **384**
  gypsoides, 388, 1275
  madurœ, 376 *et seq.*, **383**, 1275
  muris, 381, **385**, 939, 1277, 1910
  necrophorus. *See Fusiformis*
  putorii, 387
  somaliensis, 387
  variabilis, 388
Actinomycetes, antibacterial substances formed by, 175
Actinomycetin, 176
Actinomycin, 176
Actinomycosis, **1269–1278**
  differentiation of from actinobacillosis, 1280
  Drusen in, 1270, 1272
  due to *Actinomyces bovis*, 1270–1274
    in cattle, 1271
  due to aerobic *Actinomyces*, 1270, **1275–1276**
    cattle farcy, 1276
    in goats and horses, 1276
    in man, 1275
    Madura disease, 1275

Actinomycosis, due to facultative aerobic *Actinomyces*, 1276–1278
infective arthritis of mice, 1278
rat-bite fever, 1276
in swine, 1271
mode of infection in, 1271
Activated sludge method in sewage purification, 2033
Active immunity. *See* Immunity, active
Adaptive enzymes in bacteria, 293
Adhesion phenomenon, 912, 1833
Adjuvant substances for enhancement of antigenic response, 1115, 1116, 1123
Adsorption in antigen-antibody reactions, 199, **216–219**, 226, 240, 273–275
Aerobacter. *See Bacterium ærogenes*
Aerobes, obligatory, 70, 360
enzyme systems of, 70
Aerobic and anaerobic incubation, use of in obtaining pure cultures, 354, 360
Aerobic spore-bearing bacilli. *See Bacillus*
Aerobiosis, 70 *et seq.*
in identification of bacteria, 360
Aerosols, 2007–2009
African horse sickness, 1965
Agalactia, contagious, of goats, 945, 1867
Agar, 10
Agglutinating serum, use of in obtaining pure cultures, 355
Agglutination, 211. *See also* Antigen-antibody reactions *and under individual diseases*
Agglutinins. *See also* Antigen-antibody reactions
absorption of, by bacteria, 212, 273
cold, in virus pneumonias, 1877
formation of in response to infection, 1120
to primary stimulus, 1106
to repeated injections, 1113
natural, 1085 *et seq.*
for various organisms. *See under individual diseases*
protective action of, 1058
Agglutinogens, 195. *See also* Antigens, bacterial
Aggressins, bacterial, 1068–1074
antigens, relation of to, 1072
anti-opsonic effect of, 1069–1071
pneumococcal polysaccharides as, 1678
polysaccharide haptens as, 1072
Air, bacteriology of, **2002–2010**
contamination of, 2004
droplet infection of, 1312, 2003
infection of, measures for controlling, 2005–2010
liquid, effect of on bacteria, 112
Akiyami, 1834
Alastrim, 1884, 1889
Alcohol, effect of on immunity, 1209
Alcoholized T.A.B. vaccine, 1553
Alcohols, effect of on bacteria, 132
Alexine, 193, 196
Alimentary tract, bactericidal mechanisms in, 1020
normal flora of, 1986
importance of, 1989
origin of, 1989
suppurative lesions of, 1497
Alkalies, effect of, on bacteria, 120
on bactericidal action of gastric juice, 1021–1023
Allantiasis. *See* Botulism
Allergy, **1160–1168**
bacterial, 1162
relation to immunity, 1163, 1201, 1330
infection-immunity in, 1165
Koch's phenomenon as example of, 1161, 1326
tuberculin reaction, as example of, 1161, 1326
virus diseases, in relation to, 1241
Alpha rays, effect of on bacteria, 110
α-hydroxyphenazine, 173
Aluminium salts, effect on antigenic stimulus, 1115
Alum-precipitated toxoid in diphtheria immunization, 1393
Amboceptors, 198, 225, 228
Amino-acids, as essential nutrients for bacteria, 65
rôle of in bacterial nutrition, 56, 62
Amino-acridine compounds in chemotherapy, 172
Ammonia, methods of examination for, 369
Anæmia, infectious, of dogs, 904, 1798
of horses, 1837, 1966
of rats, 904, 1797
Anaerobes, enzyme systems of, 70
facultative, 70, 360
obligatory, 70, 360
Anaerobic bacilli, species of. *See Fusiformis and Clostridium*
Anaerobic infections of animals, 1778–1783
black disease, 1780
Cl. gigas in, 1781
relation of liver flukes to, 1781
blackleg, 1778
braxy, 1779
German type, 1780
Northern type, 1779
due to *Cl. welchii*, 1781–1783
enterotoxæmia, infectious, of sheep, 1782
lamb dysentery, 1781
pulpy kidney disease of lambs, 1782
" struck " in sheep, 1782
gas gangrene, 1778
malignant œdema, 1778
Anaerobic spore-bearing bacilli. *See Clostridium*
Anaerobic streptococci, 596, 1484, 1772, 1996
Anaerobiosis, 70 *et seq.*
in identification of bacteria, 354, 360
possible relation of hydrogen peroxide to, 72
rôle of oxidation-reduction potential in, 71
Anamnestic reaction, 1122
diagnosis of enteric fever in inoculated persons in relation to, 1539
Anaphylactic shock, **1136–1151**
acetyl-choline in, 1150
anaphylactoid shock, relation of to, 1144
anaphylatoxin in, 1143
desensitization in, 1142
different antisera, effectiveness of in, 1140

Anaphylactic shock, haptens, rôle of in, 1141
histamine shock, relation of to, 1144, 1148
humoral hypothesis of, 1143
in the dog, 1138
in the guinea-pig, 1137, 1147
in the rabbit, 1138
in various animals, 1139
induction period in, 1136
inhibition by antihistamine sera, 1150
by histaminase, 1150
by normal sera, 1148
mechanism of, 1142–1151
cellular hypothesis of, 1145
Schultz-Dale technique in study of, 1146
passive sensitization in, 1140
peptone shock, relation of to, 1144
reversed passive anaphylaxis in, 1140
sensitization to, factors effecting, 1136
Anaphylactoid shock, 1144
Anaphylatoxin, 1143
Anaphylaxis, **1136–1151,** 1156. *See also* Allergy, Anaphylactic shock *and* Hypersensitiveness
Arthus' phenomenon in, 1151
genetic factors in, 1159
local reactions in, 1151, 1156
relation of to immunity, 1146, 1164
Anatoxin, 1392
Angina, Vincent's, 914, 1810
Angular conjunctivitis, 898
Aniline dyes, effect of on bacteria. *See* Dyes
Animal diseases. *See under name of disease and species of animal*
Animal poxes. *See* Poxes, animal
Animals, anaerobic infections of. *See* Anaerobic infections of animals
Anions, effect of on bacteria, 124
Antagonizers of chemotherapeutic agents, 159, 161, 165, 166
Anthrax, **1730–1744**
Ascoli test in, 1738
chemotherapy in, 1743
cutaneous, susceptibility to, 1183, 1184
diagnosis of, 1737
experimental production of in animals, 1736
in animals, 1731, 1735
infection of milk in, 1735, 1740
in man, 1733, 1735
local immunity to, 1184
mode of infection and spread, 1732
by earth-worms, 1732
by flies, 1733
by food-stuffs, 1732
natural immunity to, 1739
prevention of, 1740
serum treatment of, 1743
symptomatic. *See* Blackleg
vaccination against, 1740
wool-sorters' disease in, 1733, 1734
Antibacterial immunity. *See* Immunity, antibacterial
Antibacterial index, 162
Antibacterial substances, 155, 172
from bacteria, 173, 174
from moulds, 175
Antibacterial substances, susceptibility to in relation to Gram's stain, 38, 170
Antibiotic agents, 155, 175
Antibodies. *See also under* Antigen-antibody reactions
availability in tissues of host, 1058
blocking, in allergic reactions, 1159
distribution of in tissues, 1058
electrophoretic fractionation, 242, 246, 248, 1030, 1092, 1385
fate of, after injection, 1124
formation of, 1101 *et seq.*
adjuvants, effect of on, 1115
agglutinins O, formation of after administration of antigen by mouth, 1117
anamnestic reaction in, 1122
as evidence of specific infection, 1002
avitaminosis, effect of on, 1192, 1198, 1204
blockade, effect of on, 1103
by synthesis in contact with antigens, 1127, 1128
different antigens, in response to, 1108
dispersal of inoculum, effect of on, 1115
dosage, effect of on, 1107
in skin, 1102
in lymphocytes, 1105
in tissue cultures, 1105
in various organs, 1102, 1104
in vitro, 1128
local, 1101
mechanism of, 1127
metallic salts, effect of on, 1115, 1122
multiple antigens, in response to, 1109
negative phase in, 1116
non-specific stimulus in relation to, 1120
oral administration of antigen in response to, 1117
physiological fate of, 1127
pneumococcal polysaccharides, in response to, 1684
primary stimulus in response to, 1106
protective action of *in vivo*, conditions of, 1057
repeated injections, effect of on, 1113
reticulo-endothelial system, rôle of in, 1102
secondary stimulus in response to, 1110
spleen, rôle of in, 1103
sulphonamides, effect of on, 1213
synthesis *in vitro* of, 1128
transference of *via* colostrum, 1077, 1088
via placenta, 1077, 1088
various factors, effect of on, 1104
various tissues, rôle of in, 1105
virus injections in, 1241
volume of inoculum, effect of on, 1114
general properties of, 194, 242
" heavy," 245
heterophile. *See* Forssman antibody

Antibodies, modification of, by chemical means, 247
by enzymes, 247
by physical means, 247
molecular weight of, 243, 245
monospecific, homogeneity of, 249
natural, **1075–1097**
age in relation to, 1091
antibacterial antibodies, 1085, 1092–1096
agglutinins, 1085 *et seq.*
Brucella infections, 1699
in latent *Salmonella* infections, 1533
bactericidins, 1086
complement-fixing, 1086
immunological significance of, 1095
origin of, 1092–1095
antitoxins, 1075
in blood of normal horses, 1084
immunological significance of, 1084
origin of, 1076–1084
environment in relation to, 1080 *et seq.*
rate of development under different conditions, 1083
viricidal, 1226
possible inclusion of antigens in, 1127
protein nature of, 242 *et seq.*
purification of, 243
reagins, relation of to, 1157
serum-globulins, relation of to, 244
site of formation of, **1101–1106**
unity or diversity of, 248–252, 285
valency of, 250
various, names of, 195
Antibody-forming apparatus, **1101–1131**. *See* Antibodies, formation of
Antibody globulin, 242
varieties of, 245
Anti-endotoxins, 1008
failure of law of multiple proportions in action of, 1009
Antiformin, use of in obtaining pure cultures, 354, 418, 1321
Antigen-antibody reactions, **192–265**
adsorption in, 199, **216–219**, 226, 240, 273–275
agglutination, 211
cohesive force in relation to, 214
electric charge in relation to, 213
electrolytes in relation to, 213
in diagnosis. *See under individual diseases*
optimal antigen—antibody ratio in relation to, 212
potential difference in relation to, 214
tests of lattice hypothesis in, 221
alexine, 193, 196
antigen and antibody, combination of, in varying proportions, 205, 216, 219, 227 240
antigenic specificity, surface structure in relation to, 263
Arrhenius and Madsen's theory of, **199**, 239
Antigen-antibody reactions, bactericidins, 228
bacteriolysis, 192, 228
Neisser-Wechsberg phenomenon in, 228
Pfeiffer's reaction, 192, 1050, 1425
bacteriotropins, 194, 235
complement, relation of to, 236
electric charge in relation to action of, 235
relation of to opsonins, 235
Bordet's theory of, 199
capsular swelling reaction, 241
combination in varying proportions, 205
complement, 193, 196
nature of, 229
unity or diversity of, 229
complement fixation, 232
in diagnosis. *See under individual diseases*
optimal conditions for, 233
relation of to precipitin reaction, 233
differences in antigenic structure in relation to, 285
efficacy of *in vivo*, 1061
Ehrlich's theory of, 196
electrolytes, effect of on, 200, 207, 211, **213**, 226
Freundlich isotherm in relation to, 218
hæmolysis, 193, 224
supposed rôle of amboceptors in, 225
temperature, effect of, on, 227
haptens, inhibition of precipitation by, 257
hydrophile and hydrophobe states in relation to, 209
lattice hypothesis of, 208, 220
law of multiple proportions in, 217
mass law in, 219
microscopic appearance of specific aggregates, 223
miscellaneous manifestations of, 240
nature of union in, 263
opsonins, 194, 235
complement, relation of to, 236
electric charge in relation to action of, 235
relation of to bacteriotropins, 235
optimal antigen: antibody ratio in relation to, 201 *et seq.*, 212
relation of constant-antigen ratio to constant-antibody ratio, 203–207
relation of to chemical equivalence, 204
precipitin reaction, 200
effect of various factors on, 200
electrolytes, effect of, on, 200
in standardization of antipneumococcal serum, 1681
optimal antigen-antibody ratio in relation to, 201
relation of to complement fixation, 233
tests of lattice hypothesis in, 221
qualitative aspects of, 242–265
quantitative aspects of, 201–241
reversibility of, 216
effect of salts on, 214
" side-chain " theory of, **196**

Antigen-antibody reactions, specificity of, 252
terminology employed in relation to, 194 *et seq.*
toxin-antitoxin reaction, 238–240
antitoxin, unit of, 238
Danysz phenomenon in, 216, 240
Ehrlich's phenomenon in, 239
epitoxoids in relation to, 239
toxin, L_0 and L_+ doses of, 239
Minimal Lethal Dose of, 238
unit of antitoxin, 238
" two-stage " hypothesis of, 208
unitarian hypothesis of, 248
unity or diversity of antibodies, 248, 252
Antigenic structure of bacteria, 273–285. *See also under individual species*
bacterial variation in relation to, 276
bacteriophage activity in relation to, 338
chemical methods in determination of, 279
classification in relation to, 314
determination of by selective absorption of agglutinins, 273
different antigen-antibody reactions in relation to, 285
fallacies in determination of, 274, 275, 281
flagellar antigens, 276, 283, 284
group and specific phases of, 279
Forssman antigen in, 282, 1090
H antigens, group and specific phases of, 278
heterophile antigen in, 282
localization of antigens in bacterial cell, 283
O antigens, 277
optimal proportions, in determination of, 276
roughness in relation to, 277, 285
sharing of antigens by different bacteria, 282
smoothness in relation to, 277, 285
somatic antigens, 276, 283
Antigenicity, of denatured protein, 261
of gelatin, 260
of proteins, 259
rôle of carbohydrate in, 261
Antigens. *See also* Antigenic structure of bacteria *and* Antigen-antibody reactions
atopens, relation of to, 1154 *et seq.*
bacterial. *See under* Antigenic structure of bacteria
aggressins, relation of to, 1071
bacteriophage, in relation to, 338
endotoxins, relation of to, 1008
relation of to active immunization, 1055
basis of antigenicity of, 259
basis of specificity of, 252
chemical nature of in relation to stimulation of antibody production, 1119
different, effect of simultaneous injection of, 1109
filtrable viruses, of, 1240
Forssman, 1089
in bacteria, 282, 1090
general properties of, 194
Antigens, haptens, 258
aggressive action of in pneumonia, 1071, 1679
anaphylaxis, rôle of in, 1141
antibody formation, stimulation of, by, 1119, 1684
bacterial polysaccharides as, 258
complex, 258
in filtrable viruses, 1237
in Forssman antigens, 1090
in human group A substance, 1090
inhibition of precipitation by, 257
relation of to complete antigens, 258
simple, 258
masked, 262
nature and properties of, 252
organ specific, 252
persistence in body, effect on immunity of, 1129
possible inclusion of in antibodies, 1127
proteins in relation to full antigenicity of, 260, 1119
species, specific, 252
synthetic, 253–256
diazo reaction in preparation of, 253
inducing type-specific immunity against pneumococci, 1054
valency of, 262
various, names of, 195
Antipneumococcal immunity, experimental, in rabbit, 1037, 1048–1052
Antisepsis, Lister's studies on, 11
Antitoxic immunity, **1029–1033.** *See* Immunity, antitoxic
Antitoxin. *See also under individual diseases*
avidity of, 1030
natural, 1075 *et seq.* *See* Antibodies, natural
route of administration of, effect of, 1124, 1763
unit of, 238
Antiviral antibodies, 1237
Antiviral immunity. *See* Immunity, antiviral
Appendicitis, 1497
bacteriology of, 1497
isolation of *Fusiformis* from, 477 *et seq.*
Arrhenius and Madsen's theory of antigen-antibody reactions, 199, 239
Arsenic compounds in chemotherapy, 155, 156, 171
Arsenic-fast strains of spirochætes in relapsing fever, 1808
Arthritis in rheumatism, 1509 *et seq.*
in joint ill, 676, 1284, 1496
infective of mice, 386, **1278**
purulent, 1496
Arthus' phenomenon, 1151
Ascococcus mesenterioides, 627
Ascoli's thermoprecipitin reaction, 1738
Aspergilli, 175
Aspergillic acid, 177
Asterococcus canis, 946
mycoides, 940
Asthma, hypersensitiveness in relation to, 1153
Atopens, 1154 *et seq.*
simple compounds as, 1155
Aujesky's disease, 1941

Australian X encephalitis, 1918
Auto-disinfecting mechanism of alimentary tract, 1020, 1988
of conjunctivæ, 1025
of genital tract, 1025
of nose and naso-pharynx, 1023
of skin, 1019
Autotrophic bacteria, 61
Autoxidizable substances in bacterial metabolism, 48
Autumn diarrhœa in cattle, 1720
fever of Japan, 1828, 1836
fever of Queensland, 1828, 1837
Avian spirochætosis, 1808
Avidity of antitoxin, 1030
Azotobacter, 497–499
agilis, 499
beijerincki, 497
chroococcum, 497, 498
vinelandii, 497
vitreus, 497
woodstowni, 497

B.C.G., 1306, 1331, 1337, 1347
B encephalitis, 1917
B virus of myelitis, 1921
Babes-Ernst granules, 23
in *C. diphtheriæ*, 449
in *Neisseria*, 532
Bacillæmia. *See Bacteræmia*
Bacillary hæmoglobinuria of cattle, 891
Bacillary white diarrhœa of chicks, 1555
Bacillus, Bang's abortion. *See Brucella abortus*
Bang's necrosis. *See Fusiformis necrophorus*
of Abel-Loewenberg, 494, 667, 1789
of Achalme, 886
of Boas-Oppler, 750, 757, 764
of Bordet-Gengou. *See Hæmophilus pertussis*
of Calmette-Guérin (B.C.G.), 1306, 1331, 1337, 1347
of Danysz. *See Salmonella enteritidis*
of Deneke. *See Vibrio*
of Döderlein, 750 *et seq.*, 757, 1996
of Ducrey, 791 *et seq.*, 807, 1790
of Finkler-Prior. *See Vibrio*
of Flexner. *See Shigella*
of Friedländer. *See Bacterium*
of Gaertner. *See Salmonella enteritidis*
of Glässer. *See Salmonella typhi-suis*
of Grigoroff, 764
of von Hibler (IX). *See Clostridium tertium*
of Hirschfeld. *See Salmonella paratyphi C*
of Hiss, 689
of Hofmann. *See Corynebacterium*
of Johne. *See Mycobacterium*
of Koch-Weeks, 791 *et seq.*, 807, 1496
of Massauah. *See Vibrio*
of Massol. *See Lactobacillus bulgaricus*
of Morax-Axenfeld, 898
of Morgan. *See Proteus morgani*
of Nasik. *See Vibrio*
of Nocard, 1873
of Perez, 494
Bacillus of Petit, 899
of Preisz-Nocard. *See Corynebacterium ovis*
of Rodella (III). *See Clostridium tertium*
of Schmitz. *See Shigella*
of Schmorl. *See Fusiformis necrophorus*
of Shiga. *See Shigella*
of Sonne. *See Shigella*
of Sordelli. *See Clostridium bifermentans*
of Voldagsen. *See Salmonella typhi-suis* var. *voldagsen*
of Welch. *See Clostridium*
of Whitmore. *See Pfeifferella*
of leprosy. *See Mycobacterium lepræ*
of mouse septicæmia. *See Erysipelothrix muriseptica*
of ozæna. *See* Ozæna
of rat leprosy. *See Mycobacterium* of rat leprosy
of soft chancre, 791 *et seq.*, 807, 1790
of sputum septicæmia, 668
of timothy grass. *See Mycobacterium phlei*
aberdeen. *See Salmonella*
abony. *See Salmonella*
abortus. *See Brucella*
abortus-bovis. *See Salmonella*
abortus-equi. *See Salmonella*
abortus-ovis. *See Salmonella*
accra. *See Salmonella dublin*
acetobutylicum. *See Clostridium*
acidi-lactici. *See Bacterium*
acidophil-ærogenes. *See Lactobacillus*
acidophilus. *See Lactobacillus*
acnes. *See Corynebacterium*
actinoides. *See Actinobacillus*
actinomycetem-comitans. *See Actinobacillus*
adelaide. *See Salmonella*
ærofœtidus. *See Clostridium*
ærogenes. *See Bacterium*
ærogenes capsulatus. *See Clostridium welchii*
ærtrycke. *See Salmonella typhi-murium*
æruginosus. *See Pseudomonas pyocyanea*
agilis. *See Azotobacter*
alkalescens. *See Shigella*
alkaligenes. *See Bacterium*
altendorf. *See Salmonella*
amager. *See Salmonella*
ambiguus. *See Shigella schmitzi*
amersfoort. *See Salmonella*
amethystius. *See Chromobacterium*
amherstiana. *See Salmonella*
amylobacter. *See Clostridium*
anatum. *See Salmonella*
aquatilis. *See Chromobacterium*
arechavaleta. *See Salmonella*
arizona. *See Salmonella*
arthritidis muris. *See Corynebacterium*
asiaticus. *See Bacterium*
aurantiacus. *See Chromobacterium*
avisepticus. *See Pasteurella*
azophile, 499
ballerup. *See Salmonella*
bareilly. *See Salmonella*
batavia. *See Salmonella*

Bacillus beijerincki. *See Azotobacter*
berlin. *See Salmonella thompson*
berolinensis. *See Vibrio and Lactobacillus*
berta. *See Salmonella*
bifermentans. *See Clostridium*
bifermentans sporogenes. *See Clostridium bifermentans*
bifidus. *See Lactobacillus*
binns. *See Salmonella typhi-murium*
bispebjerg. *See Salmonella*
blegdam. *See Salmonella*
bonariensis. *See Salmonella*
borbeck. *See Salmonella*
botulinus. *See Clostridium*
bovimorbificans. *See Salmonella*
bovisepticus. *See Pasteurella*
brænderup. *See Salmonella*
brandenburg. *See Salmonella*
bredeney. *See Salmonella*
brevis. *See Lactobacillus*
bronchicanis. *See Hæmophilus bronchisepticus*
bronchisepticus. *See Hæmophilus*
bubalisepticus. *See Pasteurella*
buccalis muciferens, 668
buchneri. *See Lactobacillus*
budapest. *See Salmonella*
bulgaricus. *See Lactobacillus*
butyricus. *See Clostridium and Mycobacterium*
cadaveris butyricus. *See Clostridium welchii*
california. *See Salmonella*
caloritolerans. *See Clostridium*
cambridge. *See Salmonella*
canalis capsulatus, 667
canastel. *See Salmonella*
capitovalis. *See Clostridium*
capsulatus, 668
cardiff. *See Salmonella*
carnis. *See Clostridium*
carrau. *See Salmonella*
casei. *See Lactobacillus*
caucasicus. *See Lactobacillus*
caviæ. *See Pseudomonas*
cavisepticus mobilis, 787
centrosporogenes. *See Clostridium*
cerro. *See Salmonella*
ceylonensis, 685
chaco. *See Salmonella*
chauvœi. *See Clostridium*
chester. *See Salmonella*
choleræ. *See Vibrio*
choleræ-suis. *See Salmonella*
chroococcus. *See Azotobacter*
claibornei. *See Salmonella*
cloacæ. *See Bacterium*
cochlearius. *See Clostridium*
cœruleus. *See Chromobacterium*
coli. *See Bacterium*
coli-anaerogenes. *See Bacterium*
coli-communior. *See Bacterium*
coli-communis. *See Bacterium*
coli-mutabilis. *See Bacterium*
columbensis. *See Bacterium*
concord. *See Salmonella*
copenhagen. *See Salmonella typhi-murium*
Bacillus coryzæ segmentosus, 450
coscoroba. *See Bacterium*
crassus. *See* Döderlein's bacillus
crassus sputigenus, 668
cuniculi, 479
cyanogenus. *See Pseudomonas*
danubicus. *See Vibrio*
danysz. *See Salmonella*
dar-es-salaam. *See Salmonella*
denitrificans. *See Chromobacterium*
denitrificans fluorescens. *See Pseudomonas*
derby. *See Salmonella*
difficilis. *See Clostridium*
diphtheriæ. *See Corynebacterium*
diphtheriæ vitulorum. *See Fusiformis necrophorus*
dispar. *See Shigella*
dublin. *See Salmonella*
ducreyi. *See Hæmophilus*
duesseldorf. *See Salmonella*
duisburg. *See Salmonella*
duplex, 899
durban. *See Salmonella*
dysenteriæ. *See Shigella*
eastbourne. *See Salmonella*
El Tor. *See Vibrio*
enteritidis. *See Salmonella*
enteritidis sporogenes. *See Clostridium welchii*
equi. *See Corynebacterium*
equirulis. *See Bacterium*
erysipelatis suis. *See Erysipelothrix rhusiopathiæ*
erysipeloides. *See Erysipelothrix*
essen. *See Salmonella*
europœus. *See Nitrosomonas*
exilis. *See Lactobacillus*
fæcalis alkaligenes. *See Bacterium*
fallax. *See Clostridium*
fermenti, 756. *See Lactobacillus*
fetus. *See Vibrio*
flavescens. *See Neisseria*
flavus. *See Nitrobacter*
flexneri. *See Shigella*
florida. *See Salmonella*
fluorescens. *See Pseudomonas*
fluorescens liquefaciens. *See Pseudomonas*
fœtidus ozænæ. *See* Perez's bacillus
fragilis. *See Fusiformis*
friedländeri. *See Bacterium*, Friedländer group of
funduliformis. *See Fusiformis necrophorus*
furcosus. *See Fusiformis*
fuscus. *See Chromobacterium*
fusiformis. *See Fusiformis* and *Bacillus*
gallinarum. *See Salmonella*
gaminara. *See Salmonella*
gärtner. *See Salmonella enteritidis*
georgia. *See Salmonella*
gigas. *See Clostridium œdematiens*
giumai. *See Bacterium*
give. *See Salmonella*
glostrup. *See Salmonella*
goettingen. *See Salmonella*

Bacillus granulosis. *See Bacterium*
grumpensis. *See Salmonella*
grünthal. *See Bacterium*
habana. *See Salmonella*
hæmoglobinophilus canis. *See Hæmophilus canis*
hæmolyticus. *See Clostridium*
hartford. *See Salmonella*
hastiforme. *See Clostridium*
heidelberg. *See Salmonella*
helcogenes. *See Vibrio*
histolyticus. *See Clostridium*
hofmannii. *See Corynebacterium*
hormæchei. *See Salmonella*
horsham. *See Salmonella*
hvittingfoss. *See Salmonella*
icteroides. *See Salmonella typhi-murium*
illinois. *See Salmonella*
immobilis. *See Bacterium*
indicus. *See Chromobacterium*
infantis. *See Salmonella*
influenzæ. *See Hæmophilus*
innutritus. *See Clostridium*
inverness. *See Salmonella*
italiana. *See Salmonella*
janthinus. *See Chromobacterium*
java. *See Salmonella paratyphi B*
javiana. *See Salmonella*
jena. *See Salmonella enteritidis*
kaapstad. *See Salmonella*
kaposvar. *See Salmonella*
kaukasicus. *See Lactobacillus caucasicus*
kentucky. *See Salmonella*
khartoumensis. *See Bacterium*
kiel. *See Salmonella*
kielensis. *See Chromobacterium*
kirkee. *See Salmonella*
koeln. *See Salmonella*
kottbus. *See Salmonella*
kunzendorf. *See Salmonella choleræ-suis*
lactis ærogenes, *See Bacterium aerogenes*
lactis erythrogenes. *See Chromobacterium*
lacunatus, 898
leguminosarum. *See Rhizobium*
lepisepticus. *See Pasteurella*
lepræ. *See Mycobacterium*
lexington. *See Salmonella*
lignieresi. *See Actinobacillus*
litchfield. *See Salmonella*
london. *See Salmonella*
loma-linda. *See Salmonella*
luteus. *See Micrococcus*
madampensis, 685
madelia. *See Salmonella*
mallei. *See Pfeifferella*
manchester. *See Shigella newcastle*
manhattan. *See Salmonella*
melaninogenicus. *See Fusiformis*
meleagridis. *See Salmonella*
melitensis. *See Brucella*
mexicana. *See Salmonella*
mikawasima. *See Salmonella*
minnesota. *See Salmonella*
mirabilis. *See Proteus*
mississippi. *See Salmonella*
moniliformis. *See Actinomyces muris*
monocytogenes. *See Erysipelothrix*
Bacillus montevideo. *See Salmonella*
morbificans bovis. *See Salmonella bovis-morbificans*
morgani. *See Proteus*
moscow. *See Salmonella*
moskau. *See Salmonella enteritidis*
mucosus capsulatus. *See Bacterium*, Friedlander group of
muenchen. *See Salmonella*
muenster. *See Salmonella*
multifermentans. *See Clostridium*
multifermentans tenalbus. *See Clostridium*
muris. *See Actinomyces and Mycobacterium*
murisepticus. *See Erysipelothrix and Pasteurella*
murium. *See Corynebacterium murium*
mycoides roseus. *See Chromobacterium*
napoli. *See Salmonella*
narashino. *See Salmonella*
neapolitanus. *See Bacterium*
necrodentalis. *See Lactobacillus acidophilus*
necrophorus. *See Fusiformis*
nephritidis equi. *See Bacterium*
new-brunswick. *See Salmonella*
newcastle. *See Shigella, and Salmonella senftenberg*
newington. *See Salmonella*
newport. *See Salmonella*
niloese. *See Salmonella*
novyi I. *See Clostridium œdematiens*
nyborg. *See Salmonella*
ochraceus. *See Chromobacterium*
odense. *See Salmonella paratyphi B*
odontolyticus I and II. *See Lactobacillus*
œdematiens. *See Clostridium*
œdematis maligni. *See Clostridium septicum*
œdematis maligni II. *See Clostridium œdematiens*
œdematis sporogenes. *See Clostridium bifermentans*
œdematoides. *See Clostridium*
onarimon. *See Salmonella*
onderstepoort. *See Salmonella*
oranienburg. *See Salmonella*
oregon. *See Salmonella*
oslo. *See Salmonella*
ovisepticus. *See Pasteurella*
ozogenes, 495
panama. *See Salmonella*
para-abortus. *See Brucella*
paracholeræ. *See Vibrio*
para-influenzæ. *See Hæmophilus*
paramelitensis. *See Brucella*
parapertussis. *See Hæmophilus*
paraputrificus. *See Clostridium*
para-Shiga. *See Shigella*
parasporogenes. *See Clostridium*
parasuis. *See Brucella*
paratyphosum. *See Salmonella*
pasteurianus. *See Lactobacillus and Clostridium*
pensacola. *See Salmonella*
pentoaceticus. *See Lactobacillus*

Bacillus perfringens. *See Clostridium welchii*
pertussis. *See Hæmophilus*
pestis. *See Pasteurella*
pestis caviæ. *See Salmonella typhi-murium*
phlegmonis emphysematosæ. *See Clostridium welchii*
phlei I and II. *See Mycobacterium*
phosphorescens. *See Vibrio*
plantarum. *See Lactobacillus*
pneumoniæ. *See Bacterium*, Friedländer group of
pneumoniæ caviarum. *See Hæmophilus bronchisepticus*
pneumosintes. *See Bacterium*
pomona. *See Salmonella*
poona. *See Salmonella*
porci. *See Erysipelothrix rhusiopathiæ*
potsdam. *See Salmonella*
pretoria. *See Salmonella*
prodigiosus. *See Chromobacterium*
proteus vulgaris. *See Proteus*
pseudomallei. *See Pfeifferella whitmori*
pseudopneumonicus, 667
pseudotuberculosis. *See Pasteurella, Corynebacterium murium, and Corynebacterium ovis*
psittacosis. *See Salmonella typhi-murium*
pueris. *See Salmonella*
puerto-rico. *See Salmonella newport*
pullorum. *See Salmonella*
pulmonum glutinosus. *See Hæmophilus bronchisepticus*
purifaciens. *See Bacterium*
putrificus. *See Clostridium*
pyocyaneus. *See Pseudomonas*
pyogenes. *See Corynebacterium*
pyosepticus equi. *See Bacterium*
radicicola. *See Rhizobium*
ramosus. *See Fusiformis*
reading. *See Salmonella*
renalis bovis. *See Corynebacterium renale*
rhinoscleromatis, 667 *et seq.*, 1789
rhusiopathiæ. *See Erysipelothrix*
rostock. *See Salmonella*
rubefaciens. *See Chromobacterium*
ruber. *See Chromobacterium*
rubislaw. *See Salmonella*
rubricus. *See Chromobacterium*
saccharobutyricus immobilis. *See Clostridium welchii*
saint-paul. *See Salmonella*
salinatis. *See Salmonella*
san-diego. *See Salmonella*
sanguinarium. *See Salmonella gallinarum*
schleissheim. *See Salmonella*
schwartzengrund. *See Salmonella*
selandia. *See Salmonella*
sendai. *See Salmonella*
senftenberg. *See Salmonella*
septique. *See Clostridium septicum*
serpens. *See Fusiformis*
shangani. *See Salmonella*
shigæ. *See Shigella*
simiæ. *See Bacterium*
simsbury. *See Salmonella*
smegmatis. *See Mycobacterium*
Bacillus solt. *See Salmonella*
sonnei. *See Shigella*
sordellii. *See Clostridium bifermentans*
sphenoides. *See Clostridium*
sporogenes. *See Clostridium*
stanley. *See Salmonella*
stercoris. *See Mycobacterium*
storrs. *See Salmonella typhi-murium*
suipestifer. *See Salmonella choleræ-suis*
suis. *See Brucella*
suisepticus. *See Pasteurella*
sundsvall. *See Salmonella*
szentes. *See Salmonella*
taksony. *See Salmonella*
tel-aviv. *See Salmonella*
tennessee. *See Salmonella*
tertius. *See Clostridium*
tetani. *See Clostridium*
tetanomorphus. *See Clostridium*
thermosaccharolyticum. *See Clostridium*
thompson. *See Salmonella*
tim. *See Salmonella newington*
tuberculosis. *See Mycobacterium*
tularensis. *See Brucella*
typhi. *See Salmonella*
typhi-exanthematici. *See Corynebacterium typhi*
typhi-flavus. *See Chromobacterium*
typhi-murium. *See Salmonella*
typhi-suis. *See Salmonella*
typhosus. *See Salmonella*
uganda. *See Salmonella*
urbana. *See Salmonella*
vaginæ enphysematosæ. *See Clostridium welchii*
vaginalis. *See* Döderlein's bacillus
vejle. *See Salmonella*
vinelandii. *See Azotobacter*
violaceus. *See Chromobacterium*
virchow. *See Salmonella*
virginia. *See Salmonella*
viscosus equi. *See Bacterium*
vitreus. *See Azotobacter*
vitulisepticus. *See Pasteurella*
voldagsen. *See Salmonella typhi-suis*
vulgaris. *See Proteus*
wehmeri, 756
welchii. *See Clostridium*
weltevreden. *See Salmonella*
wesenbergi. *See Bacterium*
whitmori. *See Pfeifferella*
wichita. *See Salmonella*
winogradskyi. *See Nitrobacter*
woodstowni. *See Azotobacter*
worthington. *See Salmonella*
xerosis. *See Corynebacterium*
zagreb. *See Salmonella*
zanzibar. *See Salmonella*
zenkeri. *See Proteus*
zopfii. *See Zopfius*
Bacillus, **838–855**
characters of, 838–842
classification of, 841
thermophilic species of, 838
albolactus, 855
anthracis, 838 *et seq.*, **842–847**
antigenic structure of, 281, 844

Bacillus anthracis, β-lysins, effect of on, 1173
pathogenicity of, 844, 1730 *et seq.*
smooth and rough forms of, 301, 843
tabulated differentiation of from pseudoanthrax bacilli, 846
variants of, 842, 845
anthracoides, 838, 846, 847
aterrimus, 850
cereus, 855
cohœrens, 855
fusiformis, 855
globigii, 850
graveolens, 855
megatherium, 839 *et seq.*, 852
mesentericus, 851
mesentericus niger, 850
mesentericus panis viscosi, 850
mesentericus ruber, 850
mycoides, 853
panis, 850
petasites, 855
polymyxa, 855
pseudoanthracis, 838 *et seq.*, 846, 847
rotans, 855
subtilis, 838 *et seq.*, 849
terminalis, 855
tumescens, 855
vulgatus, 850
Bacteræmia, 1490
after operative procedure, 1515
oral sepsis associated with, 1515
suppression of in immune animal, 1047
in normal animal, 1037
Bacteria, aggressive action of, 1068–1074
antigenic structure of, 273–285. *See also* Antigenic structure of bacteria
arrangement of, 35
autotrophic, 61
cell wall of, 27
chemical constitution of, 42–45
classification of, 310–323
epidemic strains of, 1260, 1471
examination of, methods used in systematic, 364–372
gaseous requirements of, 69
heterotrophic, 61
identification of, 357
importance of for life, 1989
infectivity of in relation to virulence, 1261–1263
intestinal, as food-stuffs, 1991
life-cycle of, 34
metabolism of, 45–74
modes of division of, 33–36
in rough variants, 300
morphology of, 16–39
nomenclature of, 310–323
nutritional requirements of, 61
reproduction of, 33, 80–99
respiration of in relation to growth, 85
size of, 16, 84, 358
staining of, 37
temperature requirements of, 360
variation of, 288–307. *See* Variation of bacteria
virulence of. *See* Virulence of bacteria
Bactericidal action of normal and immune serum, 192
Bactericidal substances, use of in obtaining pure cultures, 354
Bactericidins, 228. *See also* Antigen-antibody reactions
natural, 1086
Bactéridie, 1730
Bacteridium. *See Bacillus anthracis*
aurantiacum, 626
Bacteriolysis, 192, 228. *See also* Antigen-antibody reactions
Bacteriophage, **325–347**
absorption of, specific, by bacteria, 334
antigenic property of, 341
antiphage sera, 342
autolytic theory of, 328
bacterial precursor of, 337
carrier strains among bacteria, 345
different types of, 340
classification of, 343
differentiation of by testing against resistant bacteria, 340
by various methods, 343
discontinuous multiplication of, 333
ecology of, 345
enzyme theory of, 328
genetic theory of, 328
growth of bacteria in relation to, 327
habitat of, 327, 345
identification of bacteria by, 338
in relation to Flexner bacilli, 693
killing of bacteria by, without lysis, 334
lysogenic strains of bacteria, 346
lytic action of, 327
calcium in relation to, 333
citrate in relation to, 333
inhibition of by bacterial antigens and haptens, 339
mechanism of, 333
methods of demonstrating, 326
microscopical observation of, 336
quantitative study of, 333
metabolism of, 332
multiplicity of, 328
nature of, 327
plaques formed by, 326
different types of, 343
size of in relation to size of bacteriophage, 344
physical state of, 329
production and bacterial growth, 337
resistance to various agents, 332
resistant strains of bacteria in relation to, 340
rôle of in infection and immunity, 1215
size of, 330
specificity of, 338
relation of to antigenic structure of bacteria, 338
spores, bacterial, in relation to, 347
symbiotic with bacteria, 346
variation of, 345
variation of bacteria, as cause of, 305, 340
virus theory of, 327
Bacteriostasis, 159

Bacteriotropins, 194, 235. *See also* Antigen-antibody reactions
Bacterium, 654–681
biochemical activities of, 659–666
classification of, 654
cultural appearances of, 657
dyes, etc., action of on, 659, 705, 1527–1528
morphology of, 655
nomenclature of, 654
resistance of, 658, 2027
Salmonella antigens in, 715, 744
acidi-lactici, 665–666, 679
aerogenes, 655 *et seq.*, 679
differentiation of from *Bact. coli* and intermediate group, 660–665, 2023–2024
alkaligenes, 902
asiaticum, 677
azophile, 499
cloacæ, 661 *et seq.*, 679
coli, 655 *et seq.*, 678
antigenic structure of, 666
differentiation of from *Bact. aerogenes* and intermediate group, 660–665, 2023–2024
enzyme systems of, 59 *et seq.*
fermentative types of, 666, 2024
habitat of, 660, 661, 663
hæmolytic and non-hæmolytic types of, 1499
in food poisoning, 1606
in pyogenic infections, 1497–1503
in wound infection, 1501
lysogenic strain of, 346
pathogenicity of, 666
summarized description of, 678
toxins of, 667, 1606
coli-aerogenes group of, 660–667
in water, 2023–2024
coli-anaerogenes, 677
coli-commune, 660–661, 665, 666, 679
coli-communius, 661, 665, 666, 679
coli-mutabile, 297, 677
columbense, 678
coscoroba, 665–666
dysentery group of. *See Shigella*
equirule, 676
fæcalis alkaligenes, 902
Friedländer group of, 667–676, 680
broncho-pneumonia in relation to, 1667
capsule of, chemical constitution of, 669
classification of, 675
pathogenicity of, 674
suicide colonies of, 670
giumai, 678
granulosis, 901, 1878
grünthal, 665–666
immobile, 665–666
intermediate group of coliform bacilli, 663–667, 679, 2023–2024
khartoumense, 678
lactis aerogenes. *See Bact. aerogenes*
monocytogenes. *See Erysipelothrix*
morgani. *See Proteus*
Bacterium, mucosus capsulatus, 667. *See Bacterium*, Friedländer group of
neapolitanum, 665, 666, 679
nephritidis equi, 676
ozænæ, 670, 673, 1789
paracolon group of, 676, 681
pneumosintes, 899
purifaciens, 1499
pyosepticum equi, 676
rhinoscleromatis, 670, 673, 1789
simiæ, 902
triloculare, 655
tularense. *See Brucella*
typhi-flavum. *See Chromobacterium*
viscosum equi, 676
wesenbergi, 678
Bacteroides, 477, 751, 755
funduliformis, 479
Bail's hypothesis of limiting population density, 94
Balantidium coli, dysentery caused by, 1561
Bang's abortion bacillus. *See Brucella abortus*
Bang's necrosis bacillus. *See Fusiformis necrophorus*
Barbone of buffaloes, 1647
Bartonella, 903
diseases due to, 1796–1798
bacilliformis, 903
in Oroya fever, 1797
canis, 904
in infectious anæmia of dogs, 1798
muris, 903, 904
in infectious anæmia of rats, 1797
Bats, in relation to rabies, 1935
Benzene, effect of on immunity, 1209
Beta lysins, 1173
rays, effect of on bacteria, 110
β-alanine as bacterial vitamin, 67, 164
Bile, differential effect of on growth of streptococci, 572
Bile-solubility of pneumococcus, 571
Biochemical reactions of bacteria, methods of examination for, 367–369
variation in, 292
Bios hypothesis of lag, 84
Biotin as bacterial vitamin, 69
Bitter's medium for fermentation test, 709
Black disease, 882, 1780
Black rot of eggs, 647
Blackleg, 884, 1778
Blackquarter, 1778
Bleeding, effect of on antibody formation, 1122
Blennorrhœa, inclusion, 1880
Blockade, effect of on antibody formation, 1103
Blood, anaphylactic reaction in rabbits, 1149, 1151
bacteria normally present in, 1998
bactericidal power of, non-specific induction of increase in, 1175
Blood capillaries, permeability to foreign matter, 1042
cerebral, permeability to foreign matter, 1060, 1754, 1764
Blood culture. *See under individual diseases*
groups, human, 1087
in relation to Schick reaction, 1079
Rh factor in, 1088

Blood stream, removal of bacteria from, 1037, 1047. *See also* Immunity, antibacterial
Blue-tongue of sheep, 1967
Boas-Oppler bacillus, 750, 757, 764
Bollinger's granules, 1492
  inclusion bodies, 959, 1896
Bone taint, 873
Bones and joints, suppurative lesions in, 1496
Boosting dose. *See* Secondary stimulus
Bordet-Gengou bacillus. *See Hæmophilus pertussis*
Bordet's theory of antigen-antibody reactions, 199
Borna disease, 1921
Bornholm disease, 1960
Borrel bodies, 959, 1896
Borrelomycetales, 940
Botriomycosis, 1270, 1271, 1492
Botulism, **1612–1623**
  diagnosis of, 1617
  distribution of *botulinum* in nature, 1615
  epidemiology of, 1613
  factors influencing development of *Cl. botulinum* in canned foods, 1616
  in animals, 1619–1623
  mode of infection of food in, 1615
  prophylaxis and treatment of, 1618–1619
  type of food causing, 1614
Boyd's dysentery bacilli, 685 *et seq.*
Bradsot. *See* Braxy
Branching forms of bacilli, 33
Braxy, 1779
  German type of, 1780
  meadow type of, 1780
  Northern type of, 1779
  stall type of, 1780
Brazilian spotted fever, 1847
Breast-feeding in relation to infantile diarrhœa, 1582, 1586
Brilliant green, in cultures from enteric fever, 1527, 1528
Brill's disease, 1841, 1842
Broncho-pneumonia, 1666
  secondary, 1666–1668
  virus, 1876
Brucella, **814–834**
  antigenic structure of, 822
  band phenomenon in agar-shake cultures of, 819
  biochemical properties of, 821
  carbon dioxide requirements of, 818
  chemical fractionation of, 825
  classification and identification of, 830
    tabulated, 831
  crystal formation in cultures of, 817
  cultivation of in presence of dyes, 820, 1714
  cultural reactions of, 816
  differentiation of, on Petragnani's egg medium, 817
  growth of in sealed tubes, 819
  growth requirements of, 818
  H_2S production by, 822
  infections, **1692–1722**. *See also* Undulant fever, Contagious abortion in cattle, *and* Tularaemia
Brucella, infections of cattle, 1708
    of cats, 1719
    of dogs, 1719
    of fowls, 1719
    of goats, 1695, 1697, 1706
    of horses, 1718
    of man, 1692
    of rats, 1720
    of sheep, 1697, 1706
    of swine, 1702, 1717
  in Hodgkin's disease, 1981
  morphology of, 816
  nomenclature of, 815
  pathogenicity of, 826
  relation of to *Pasteurella*, 825
    to *Pfeifferella*, 490, 825
    to *Proteus*, 825
  resistance of, 821
  staining of, 816
  variation of, 829
  abortus, 814 *et seq.*, **832**
    institutional outbreaks due to, 1701
    isolation of from milk, 1713
    pathogenicity of for small animals, 829
    undulant fever due to, 1697
  bronchiseptica. *See Hæmophilus bronchisepticus*
  melitensis, 814 *et seq.*, **831**
    infection of cattle, 1697, 1710, 1711
    pathogenicity of for small animals, 828
    undulant fever due to, 1693
  para-abortus, 815, 823
  paramelitensis, 815, 822, 823
  parasuis, 815, 823
  suis, 814 *et seq.*, **833**
    infection of cattle, 1710, 1711
    pathogenicity of for small animals, 829
    undulant fever due to, 1702
  tularensis, 814, 815, **833**
    disease in man due to, 1720
    relation of to *Pasteurella*, 815
Brucellergin, 1705
Brucellin test in diagnosis of contagious abortion in cattle, 1713
  of undulant fever, 1705
Bubo, climatic, 1869
Buffaloes, osteomyelitis of, 1781
Bulbar paralysis in cattle, 1621
  due to pseudorabies virus, 1941
Bullis fever, 1846
Bumble-foot of chickens, 1349
Butter bacillus. *See Mycobacterium butyricum*
  in relation to undulant fever due to *Br. abortus*, 1701
Butyribacterium, 756

Calcium as kataphylactic agent, 1206, 1750, 1773
  effect of on spore formation, 291
  inhibition of pigment formation by, 635
  relation of to bacteriophage activity, 333
  rôle of in tetanus infection, 1206, 1750
Calculi salivary, *Actinomyces* in, 1272

Calf diphtheria, 479, 1787
Calmette-Guérin's bacillus (B.C.G.), 1306, 1331, 1337, 1347
Calves. *See* Cattle
Canary-pox, 953, 1896
Canine typhus of Lukes, 1837
Capillaries, cerebral, permeability of, 1060, 1754, 1764
permeability of, 1042
Capsules, bacterial, 29
aggressive action of bacteria, in relation to, 1072
hyaluronic acid in, 1072
in relation to virulence, 300, 575, 842, 1072
Carbohydrate constituents of bacteria, 44
metabolism of bacteria, 51
Carbohydrates, action of bacteria on, 52–55
protein-sparing effect of in bacterial metabolism, 57
synthesis of, by bacteria, 55
Carbon dioxide, as essential nutrient for bacteria, 61, 69
use of in blood culture, 1491, 1703
Carbon dioxide : hydrogen ratio, in differentiation of coli-ærogenes group, 661
in growth of *Brucella*, 818
rôle of in bacterial growth, 69
Carboxydomonas oligocarbophila, 505
Carrier epidemic, 1251. *See also* Carriers *under individual diseases*
Carrier types of pneumococci, 1673
Carriers, bacterial. *See under individual diseases*
herd infection in relation to, 1246
in experimental epidemics, 1253
Catalase, methods of examination for, 369
protective action of in relation to hydrogen peroxide, 72
Cataphoresis of bacteria, 213
of filtrable viruses, 950
of serum proteins, 242, 246, 248, 1030, 1092, 1385
Catarrh, malignant, of cattle, 1966
Cathode rays, effect of on bacteria, 108
Cations, effect of on bacteria, 123
Cats, *Brucella* infection in, 1719
distemper in, 1877
enteritis in, 1963
tuberculosis in, 427, 429, 1343
Cattle, actinomycosis in, 1271, 1276
anaerobic infections of. *See* Anaerobic infections of animals
autumn diarrhœa in, 1720
bacillary hæmoglobinuria of, 891
blackleg in, 884, 885, 1778
botriomycosis in, 1492
botulism in, 1621
Brucella infections of, 1708–1716
with *Br. abortus*. *See* Abortion, contagious, in cattle
with *Br. melitensis*, 1697, 1710, 1711
with *Br. suis*, 1710, 1711
bulbar paralysis of, 1621
cow-pox of, 1888
diphtheria in, 1390
diphtheria of calves due to *F. necrophorus*, 1787
Cattle, diphtheroid infections of, 458–462, 1403
dry bible of, 1621
ephemeral fever of, 1966
epizootic pneumonia of calves, 391, 1281
farcy of, 387, 1276
ictero-hæmoglobinuria of, 1621
impaction paralysis of, 1621
influenzal pneumonia of calves, 1662. *See also* Scours
Johne's disease of, 441, 1363
lamziekte of, 880, 1621
malignant catarrh of, 1966
mastitis in, 1493. *See* Mastitis in cattle
Midland disease of, 1621
plague, 1965
pleuropneumonia of, 939, 1647, 1867
pneumonia of calves due to *Actinobacillus actinoides*, 1281
pyelitis in, 461
pyelonephritis in, 1500
red-water disease of, 891
Salmonella infections of, 1600
scours in, 527, 667, 1662. *See also* Influenzal pneumonia of calves
tuberculosis in, 1317, 1342–1348
Cellobiose, in differentiation of coli-ærogenes group, 664
Cellular hypothesis of anaphylactic shock, 1145
immunity, rôle of in virus infections, 1234
reactions in bacterial infection, 1007
Cellulitis, 1493
anaerobic, 1771
Centrifugation, factors affecting, 953
Centrifuges, types of, 953
Cerebrospinal meningitis. *See* Meningitis
Chancre, hard, 1812 *et seq.*
soft, 1790
Cheese in relation to typhoid fever, 1542
to undulant fever, 1701
Chemical changes produced by bacteria, 51 *et seq.*
Chemical constitution of bacteria, 42
Chemistry of bacterial antigens. *See under individual organisms*
Chemotaxis in pyogenic infections, 1007
Chemotherapeutic activity, acidic or basic groups, 170
and relation to chemical structure, 156, 169
in the sulphonamide series, 167
as interference with essential metabolites, 162
surface activity and chain length, 170, 171
Chemotherapeutic agents, acquired resistance of micro-organisms to, 156, 160, 172, 181
antagonizer—inhibitor systems, 159, 161
nicotinamide and sulphathiazole, 165
nicotinic acid and pyridine sulphonamide, 163
pantothenic acid and pantoyltaurine, 164
p-aminobenzoic acid and the sulphonamides, 161
thiamin and pyrithiamin, 164
antagonizers of, 159, 161, 165
as inhibitors of bacterial growth, 159

Chemotherapeutic agents, cross-resistance of micro-organisms to, 161, 172
gramicidin, 174
penicillin, 179
sulphonamide compounds, 157
sulphone compounds, 171
Chesney's hypothesis of lag, 84
Chicken-pox, 1901
Chickens. *See* Fowls
Chick-Martin method of standardizing disinfectants, 147
Chicks, bacillary white diarrhœa in, 1555
Chlamydozoa, 949
Chlorine, effect of on bacteria, 128, 149, 2030
Cholecystitis, 1497. *See also* Gall-bladder, infection of
Cholera, **1418–1428**. *See also Vibrio choleræ*
diagnosis of, 1424
Pfeiffer's reaction in, 1425
epidemiology of, 1421
examination of suspected convalescents from, 1426
experimental reproduction of, 522, 1420
healthy carriers in, 1424
laboratory infections in, 1420
mode of spread in, 1422
of fowls, 1646
prophylaxis and treatment of, 1426
by bacteriophage, 1427
by chemotherapy, 1428
by serum therapy, 1428
by vaccination, 1426
Cholera-red reaction, 516
Choriomeningitis, lymphocytic, 1932
pseudo-lymphocytic, 1932, 1934
swineherds' disease, 1932, 1934
Chromobacterium, **631–640**
group forming pink or red pigment, 635
forming violet pigment, 634
forming yellow or orange pigment, 637
pigment formation by, 633 *et seq.*
amethystium, 635
aquatile, 631, 637
aurantiacum, 639
cæruleum, 635
denitrificans, 639
fuscum, 639
indicum, 635, 637
janthinum, 635
kielense, 635, 637
lactis erythrogenes, 635
mycoides roseum, 635
ochraceum, 639
prodigiosum, 631 *et seq.*, 636
rubefaciens, 635
ruber, 635
rubricum, 635
typhi-flavum, 637, 638
violaceum, 631 *et seq.*, 634
Chromogenesis. *See* Pigment formation
Circulation of blood, removal of bacteria from, 1037, 1047
Citrate, relation of to bacteriophage activity, 333
in differentiation of coli-ærogenes group, 663
Citrinin, 177
Cladothricosis, 1270
Cladothrix asteroides. *See Actinomyces*
Classification of bacteria, **310–323**
antigenic structure in relation to, 314
criteria of, 311
nomenclature in, 313
Clavacin, 177
Clavatin, 177
Clavelée, 1895
Claviformin, 177
Climatic bubo. *See* Lymphogranuloma inguinale
Clostridium, **858–893**
antigenic structure of, 864
biochemical reactions of, 863
classification of, 871
tabulated, 872, 874–875
cultural reactions of, 860
hæmolysin production by, 863
in appendicitis, 1497, 1777
infections due to, 1746–1783
metabolism of, 862
morphology of, 859
pathogenicity of, 869
resistance of, 861
thermophilic species of, 872
toxin formation by, 865–869
aerofœtidum, 872, **890**
acetobutylicum, 162, 872
bifermentans, 890, 1771–1772
botulinum, 858 *et seq.*, 879
in relation to botulism in animals, 1619
in man, 1613
pathogenicity of for laboratory animals, 869
toxin of, 866
butyricum, 858 *et seq.*, 873
capitovale, 874, 890
caloritolerans, 890
carnis, 862, 874, 890
chauvœi, 863 *et seq.*, 882, 1778
toxin of, 869
cochlearium, 861, 863, 872, 874, **891**
difficile, 874, 891, 1987
fallax, 861, 872, 874, **891**
gigas, 881, 1781
hæmolyticum, 891
hastiforme, 874, 891
histolyticum, 858 *et seq.*, 877, 1771
multifermentans tenalbum, 872, **892**
novyi I. See Cl. œdematiens
œdematiens, 861 *et seq.*, 881, 1771–1777, 1780
pathogenicity of for laboratory animals, 871
toxin of, 868
œdematoides, 890
parabotulinum, 879
in relation to botulism in animals, 1620
paraputrificum, 892
parasporogenes, 871, 872, **892**
pasteurianum. See Cl. butyricum
putrificum, 892

Clostridium septicum, 860 *et seq.*, **884**, 1771, 1778-1780
pathogenicity of for laboratory animals, 871
toxin of, 866
septique. *See Cl. septicum*
sordellii. *See Cl. bifermentans*
sphenoides, 872, 874, **892**
sporogenes, 858 *et seq.*, **876**, 1771
tertium, 862, 892
tetani, 858 *et seq.*, **888**, 1746–1767
distribution of in nature, 1748
pathogenicity of for laboratory animals, 870
toxin of, 865
tetanomorphum, 872, 874, 893
thermosaccharolyticum, 872
welchii, 858 *et seq.*, **886**
hyaluronidase of, 868, 1013
in suppurative lesions of the urinary tract, 1500
in wound infection, 1501
infections due to, 1750, 1771, 1781
infections in sheep, due to, 1781. *See also* Sheep
lecithinase of, 867, 1774
pathogenicity of for laboratory animals, 870
toxins of, 866
Coagulase, in bacterial infections, 1012
Cocco-bacillus fœtidus-ozænæ, 494, 495
Co-enzymes, rôle of in bacterial metabolism, 49
Cold, effect of on bacteria, 112
the common, 1653
vaccination against, 1654
Coli-ærogenes group, 660–667. *See also Bacterium*
differential count of, 2023
intermediate group of, 663, 2024
Coliform bacteria, differential count of, 2023
Collodion filters, 951
Collodial reactions, in relation to antigen-antibody reactions, 199, 207
Colonies, bacterial, 36
description of, 359, 364
Colony form, variation in, 299
Colorado tick fever, 1846
Colostrum, transference of antibodies in, 1077, 1092, 1782
Comma bacillus. *See Vibrio choleræ*
Common cold, the. *See* Cold, the common
Complement, 193, 196
analysis of guinea-pig, 230
analysis of human, 231
components of in relation to opsonins, 237
end-piece of, 229
fourth component of, 230
mid-piece of, 229
nature of, 229
opsonins in relation to, 235
relation to vitamin intake, 231
rôle of components in fixation, 233
rôle of in hæmolysis, 225
splitting of, 229
symbols for the components, 230
third component of, 230
unity or diversity of, 229
Complement fixation, 193, 232. *See also* Antigen-antibody reactions *and under individual diseases*
Complement-fixing antibodies. *See under* Antigen-antibody reactions
natural, 1086
Complementophilic group, 198
Concentration exponent *n* of disinfectants, 140
Conglutination test in diagnosis of glanders, 1414
Conjunctiva, normal flora of, 1998
Conjunctival sac, removal of bacteria from, 1025
Conjunctivitis, 1496
angular, 898
follicular in monkeys, 1878
swimming bath, 1880
Constant-antibody optimal ratio, 202
Constant-antigen optimal ratio, 202
Contagious abortion in cattle. *See* Abortion, contagious in cattle
Contagious pustular dermatitis of sheep, 1897
Correlated variations in bacteria, 290
Corynebacterium, **447–473**
antigenic structure of, 454
classification of, 465
diseases caused by, 1403. *See also* Diphtheria
fermentation reactions of, 452
table of, 473
morphology of, 448
pathogenicity of, 455
phosphatase production by, 453
strains of, intermediate between *C. diphtheriæ* and *C. ovis*, 459
toxin production by, 455
acnes, 462, **471**, 1403, 1492
arthritidis muris, 462
coryzæ segmentosum, 450
diphtheriæ, 447 *et seq.*
distribution of in tissues, 1369
gravis type of, 449, 450, **462**, 1370
in relation to diphtheria in man, 1370
growth requirements of, 452
hæmolysis produced by, 453
in milk, 1391, 2039
in wound infection, 1502
inhibition of, by heated blood, 451, 452
intermedius type of, **449**, **451**, **462**, 1370
in relation to diphtheria in man, 1370
lesions produced by in guinea-pigs, 457
lysogenic strains of, 346
mitis type of, **449**, **450**, **462**, 1370
in relation to diphtheria in man, 1370
morphology of, 448
pathogenicity of, 455
resistance of, 451
summarized description of, 465
tabular differentiation of types of, 463–464

Corynebacterium diphtheriæ, tellurite medium for growth of, 450–451
toxin of, 455
action of on adrenals, 458
on heart muscle, 458
gravis, *mitis* and *intermedius* types in relation to, 1375
M.L.D. of, 238, 1384
virulence of in relation to toxigenicity, 1372
virulence tests on, 1375
virulent and avirulent strains of, 458, 1375
equi, 452, 460, **469**
hofmanni, 450, 453, **466**, 473
murium, 450, 461, **470**, 473, 1404
ovis, 450, 451, 453, 459, 460, **468**, 473, 1403, 1649
diseases due to, 1403
relation of to *C. pyogenes*, 460
toxin of, 459
pseudotuberculosis murium. *See C. murium*
pseudotuberculosis ovis. *See C. ovis*
pyogenes, 451, 453, 460, **469**, 473
diseases due to, 1403, 1494, 1496, 1500
in arthritis of animals, 1496
in mastitis, 1494
in pyelonephritis of cattle, 1500
relation of to *C. ovis*, 460
renale, 458, 465, **470**, 473
typhi, 462, **472**
xerosis, 447 *et seq.*, **467**, 473
Cough plate in diagnosis of whooping cough, 1663
Counting of bacteria, 80–82
technique of total count, 80
centrifugal method, 81
Eberle's method, 80
Helber chamber method, 81
opacity method, 81
Wright's method, 80
technique of viable count, 81
dilution method, 81
plating method, 82
roll-tube method, 82
surface plate method, 82
Cow. *See* Cattle
Cow-pox, 1888
Cresols, effect of on bacteria, 133
Cristispira, balbianii, 908
Cross-infection of wounds, 1502
Crystals, mixed, formation of as analogy to antigen-antibody reactions, 264
Cultural reactions, in identification of bacteria, 359
of bacteria, terms used in description of, 364–366, 369
Culture media. *See under name of medium*
Cultures, pure, methods of obtaining, **351–357**
by aerobic and anaerobic incubation, 354
by agglutinating serum, 355
by dilution, 351
by filtration, 355
by heating, 354
by indicator media, 356
Cultures, pure, methods of obtaining, by Koch's plating method, 352
by motility, 353
by optimum temperature, 354
by pathogenicity, 356
by selective bactericidal substances, 354
by selective media, 355
by shake-tube method, 353
by single-cell methods, 357
by Veillon tube, 353
Cystitis, 1499
Cytochrome-oxidase, rôle of in bacterial oxidations, 48, 60
Cytochrome systems of bacteria and anaerobiosis, 71
Cytophilic group, 198, 225

Dale technique in study of anaphylactic shock, 1146
Danysz phenomenon, 216, 240
Danysz's bacillus. *See Salmonella enteritidis*
Dark-ground illumination, 18
in study of filtrable viruses, 952
Decline phase of bacterial growth, 83, 97
Deer-fly fever. *See* Tularæmia
Dehydrogenases, bacterial, 47, 52, 59
inactivation of, 59
Deneke's vibrio, 523, 525
Dengue fever, 1958
Dental caries, 754
Dermacentroxenus rickettsi. *See Rickettsia rickettsi*
Dermatitis, contagious of sheep, 1897
Descriptive terms for bacteria, 364–366, 369–372
Desiccation, effect of on bacteria, 111
on filtrable viruses, 961
for preserving stock cultures, 112
Desoxycholate, for lysing pneumococci, 571
Leifson's medium, 659, 1528
Deuteroalbumose, effect of on antibody formation, 1123
"Diamonds," 1283
Diaphragm, action of in intraperitoneal infections, 1040
Diaplyte vaccine, 1337
Diarrhœa borne by milk, 2049
summer, 1580, 1585, 2049. *See also* Enteritis of infancy
white bacillary of chicks, 1555
Diastase in serum, 453
Diazo reaction in preparation of synthetic antigens, 253
Dick test, 1465, 1467
age groups in relation to, 1078, 1080
native populations, in, 1081
puerperal fever in relation to, 1481
Diet as determining intestinal flora, 1990
effect of on immunity, 1190–1202
Dieudonné's medium, 515, 1424
Dilution method of counting, 81
coliform bacilli in water, 2022
use of in obtaining pure cultures, 351
Diminishing returns, law of in antibody production, 1113

Diphtheria, **1368–1403**
antitoxin, avidity of, 1030, 1385
concentrated, 1379
in blood of normal horses, 1084
of normal persons, 1075–1085
in vivo/in vitro ratio of, 1385
refined, 1379
standardization of, 1383
bacillus. *See Corynebacterium*
borne by milk, 1390, 2039, 2049
carrier: case ratio in, 1249
carriers in, 1248
avirulent strains in, 1083
effect of active immunization on, 1251
rate of clearing of, 1387
treatment of, 1386
diagnosis of, 1373
different grades of immunity to, 1250
epidemiology of, 1246–1251, 1386
experimental production of, in Schick-positive reactors, 1085
gravis, intermedius and *mitis* types of *C. diphtheriæ* in relation to 1370, 1382
immunization against,
active, 1249, 1391–1400
active and passive, 1401
effect of on carrier rate, 1399
passive, 1401
results of, 1398
in domestic animals, 1390, 1403
in pre-school children, 1399
in schools, 1248–1251
institutional epidemics of, control of, 1401
Moloney reaction in relation to, 1393, 1396
natural immunization against, 1246–1251
pathogenesis of, 1369
prophylactics against, 1391
standardization of, 1402
prophylaxis in, 1391–1403
Schick test in relation to, 1248, **1376**, 1393–1398
spread of, by animals, 1390
by dust, 1389
by fomites, 1389
by milk, 1390, 2049
susceptibility to, measurement of, 1376
toxin in relation to type of bacillus, 1372
Lf, Lo, L+, and Lr dose of, 1384
M.L.D. of, 238, 1384
M.R.D. of, 1384
Schick dose of, 1377, 1384
treatment of with antitoxin, 1378
virulence tests in, 1375
Diphtheroid bacilli. *See Corynebacterium*
in animal diseases, 1403
in human disease, 1403
Diplococcus crassus, 531, 532, **553**
intracellularis meningitidis. *See Neisseria meningitidis*
mucosus, 553
pharyngis flavus. *See Neisseria pneumoniæ*. *See Streptococcus pneumoniæ*
Disinfectants concentration exponent *n* of, 142
emulsified, 133
gaseous, 149
liquid, 149
Disinfectants, practical application of, 149
solid, 150
standardization of, 145
temperature coefficient of, 143
Disinfection, **101–150**
by acids, 114, 118
by alcohols and ethers, 132
by alkalies, 114, 120
by cathode rays, 108
by chloroxylenols, 134
by cold, 112
by desiccation, 111
by distilled water, 117
by dry heat, 112
by dyes, 134
by electricity, 107
by electromagnetic waves, 103
by essential oils, 136
by flaming, 113
by gases, 149
by heavy metals, 121
by moist heat, 113
by mould products, 172
by phenols and cresols, 133
by photodynamic sensitization, 106
by radium, 109
by Röntgen rays, 108
by salts, 120 *et seq.*
by soaps and synthetic detergents, 130
by solid disinfectants, 150
by sonic and supersonic waves, 110
by steam, 114
by sulphonamides, 157
by sunlight, 102, 104
by ultra-violet light, 102
dynamics of, 137
effect of concentration of disinfectant on, 140
of hydrogen-ion concentration on, 114, 118
of lipoid content of bacteria on, 149
of organic matter on, 134, 135
of proteins on, 149
of temperature on, 143
of variations in bacterial resistance on, 140
reaction velocity of, 137
Dispora Kaukasica. *See Lactobacillus caucasicus*
Dissociation, bacterial, 289. *See also* Variation of bacteria
Distemper of cats, 1877. *See also* Feline enteritis
of dogs, foxes and ferrets, 1961
encephalitis in, 1961
Distilled water, effect of on bacteria, 117
Division of bacteria, different methods of, 33
Döderlein's bacillus, 750 *et seq.*, 757, 1996
Dogs, anaphylactic shock in, 1138
Brucella infection of, 1719
distemper of, 1961
experimental pneumonia of, 1669
in relation to *fièvre boutonneuse*, 1848
infectious anæmia of, 904, 1798
leptospiral jaundice of, 1837
streptococcal lymphadenitis in, 1496
Stuttgart plague in, 1837
tuberculosis in, 427, 1342, 1343
typhus of Lukes in, 1837

Donovania granulomatis, 1790
Dormancy of bacteria, 98
Dose-response curve in measurement of toxicity, 995–998
Droplet infection, 1312, 2003, 2006
Droplet nuclei
fate in respiratory tract, 1024
Drug idiosyncrasies, 1153
Drug-fast micro-organisms, 156, 160, 180
Drug-fast strains of pyogenic bacteria, 1504
Drusen in actinomycosis, 375, 1270, 1272
Dry bible of cattle, 1621
Ducks, disease of due to *Cl. botulinum*, 1622
eggs of in relation to food poisoning, 1598–1599
keel disease of, 1599
Salmonella infection of, 1598
spirochætosis of, 1809
Ducrey's bacillus, 791 *et seq.*, 807, 1790
Durand's disease, 1961
Durchseuchung hypothesis of origin of natural antibodies, 1081–1083
Dust infection, 1312, 2003, 2005
Dyes, bacteriostatic effect of on bacteria, 134, 356, 659, 1527–1528
susceptibility to, of Gram-positive and Gram-negative bacteria, 134, 356
use of in differentiation of *Brucella*, 820
Dysentery, amœbic, 1561
bacillary, **1561–1576**
antiserum, use of in, 1574
definition of unit of, 1575
asylum, 1565
bacteriology of, 1566–1570
bacteriophage in treatment of, 1576
borne by milk, 1565, 2049
carriers of, 1570
chemotherapy of, 1576
diagnosis of, 1571
epidemiology of, 1562
flies in relation to, 1563
laboratory infection in man, 1571
prophylaxis of, 1573
vaccination against, 1574
winter, of calves, 527
balantidial, 1561
Dysentery group of bacteria, 685–699. *See Shigella*

Earthworms in spread of anthrax, 1732
Ectromelia, 1278, 1909
active immunization against, 1258
experimental epidemics of, 1256
Egg, dried, *Salmonella* in, 728–743, 1602
Eggs, black rot of, 647
for cultivation of viruses, 961
Salmonella infection of, 1598, 1599
Eh, 50
relation of to anaerobic growth of bacteria, 71
Ehrlich's phenomenon in toxin-antitoxin reaction, 239
method of diphtheria antitoxin standardization, 1383
theory of antigen-antibody reactions, 196
Eijkman's test, in differentiation of coli-ærogenes group, 664, 2023

Ekiri, 1568
El Tor vibrio, 518 *et seq.*, 525
Electric charge in relation to agglutination, 214, 264
to phagocytosis, 235
Electricity, effect of on bacteria, 107
Electrolytes, effect of on antigen-antibody reactions, 200
Electromagnetic waves, effect of on bacteria, 103
Electrophoresis. *See* Cataphoresis
Elementary bodies, 958, 1887
Encephalitis in animals, 1921–1925
Borna disease, 1921
horses, foxes, mice, chickens, and sheep, 1922–1924
in distemper, 1961
louping-ill, 1925
meningo-encephalitis of rabbits and guinea-pigs, 1924
Encephalitis in man, 1915–1921
Australian X type, 1918
Bwamba fever, 1920
equine type, 1918
Japanese, summer, or B type, 1917
lethargica, 1915
Russian spring-summer type, 1918
St. Louis type, 1916
secondary type, 1894, 1921
Semliki Forest type, 1920
West Nile type, 1918
Encephalitis post-vaccinal, 1894, 1921
Encephalitozoon cuniculi, 1925
Endo medium, 1528
Endocarditis, bacterial, 1514
in swine erysipelas, 1283
lenta, subacute, 1515
Endotoxins, 1008
antigens bacterial, relation of to, 1010
changes produced by in blood sugar, 1011
See also Toxins, bacterial
End-piece of complement, 229
Entamoeba histolytica, dysentery caused by, 1561
Enteric fever, **1519–1555**
active immunization against, 1550, 1554
antibody response in, 1520
bacteræmia in, 1520
bacteria causing, 1526
carriers in, 1543
detection of by Vi test, 1547
gall-bladder infections in relation to, 1522
chemotherapy in, 1555
diagnosis of, 1526–1540
by agglutination reaction, 1529
by blood culture, 1527
by cultivation from faeces, 1527
natural agglutinins in relation to, 1532
previous inoculation in relation to, 1537
effect of general sanitation on, 1548
epidemiology of, 1540
flies in relation to, 1543
mesenteric glands, infection of in, 1522
milk in relation to, 1542, 2049

Enteric fever, pathogenesis of, 1520–1525
period of disease in relation to contact infection, 1546
relapses in, 1525
route of infection in, 1523
shell fish in relation to, 1541, 2031
tonsillar infection in, 1524
treatment of with specific antisera, 1554
Vi antigen in relation to, 1525, 1547
water-borne epidemics of, 1541, 2031
Enteric infections in animals, 1555
Enteritis, feline, 1963
of animals. *See* Enteric infections
Enteritis of infancy, **1580–1590**. *See also* Enteric fever *and* Food poisoning
associated with organisms of doubtful pathogenicity, 1585
with parenteral infection, 1589
due to known pathogenic organisms, 1584
due to toxic substances of bacterial origin, 1588
epidemiology of, 1580
influence of breast-feeding on, 1582
neonatal diarrhœa in, 1583
of protozoal origin, 1588
prophylaxis and treatment of, 1589
summer diarrhœa, 1585
Enterococcus. *See Streptococcus, fæcalis* group of
Enterotoxæmia of sheep, 1782
Enterotoxic substances produced by *Salmonella*, 1607
Entiqué, 1647
Enzootic hepatitis, 1959
Enzymes, bacterial, acting on Type III pneumococcal polysaccharide, 173, 1682
adaptation of, 293
"adaptive," 293
"constitutive," 293
mutation of, 295
nature and integration of, 59
rôle of in bacterial metabolism, 46 *et seq.*
site of action of, 59
variation of, 295
Eperythrozoon, 903, 905
coccoides, 905
Epidemic strains of bacteria, 1261
Epidemiology, experimental, **1252–1264**
carriers and latent infections, 1253
epidemic strains of bacteria, 1261
genetic factors in, 1254
immunizing strains of bacteria, 1262
natural immunization, 1251
selection in, 1254
spatial distribution in, 1263
variation of bacteria in relation to, 1260
Epitoxoids, 239
Epizootic hepato-enteritis of guinea-pigs, 1960
lymphadenitis in guinea-pigs, 1495
pneumonia of calves, 391, 1281
Ergophore group, 198
Errors of random sampling, 981 *et seq.*
Erwinia, definition of, 654, 655
Erysipelas, 1479
chemotherapy in, 1485
experimental in rabbit, 1180–1182
local immunity to, 1180–1182
Erysipelas of swine. *See* Swine
Erysipeloid, 1285
Erysipelothrix, **395–403**
rough types of, 396
smooth types of, 396
erysipeloides, 399
monocytogenes, 395 *et seq.*, **401**
disease due to, 1286, 1791
muriseptica, 395 *et seq.*, 767, 1286
porci, 399
rhusiopathiæ, 171, 395 *et seq.*, **400**
disease due to, 395, 1283–1285
Erythema arthriticum epidemicum, 1277
Erythema, infective, 386, 1277
nodosum, 1332
Erythrobacillus, 631
Erythrogenic toxin of *Streptococcus pyogenes*, 591, 1465–1469
Esbach reagent for inhibiting *Proteus*, 1528
Escherichia. *See Bacterium coli*
Essential oils, effect of on bacteria, 136
Esthiomène, 1870
Ether extraction, effect of on antigen and antibody, 207
Ethers, effect of on bacteria, 132
Euglobulin. *See* Globulins
Exanthematic fever of Marseille, 1848
Exhaustion hypothesis in relation to bacterial growth, 94–97
Exotoxins, 1007–1011. *See also* Toxins, bacterial
Experimental epidemiology. *See* Epidemiology, experimental

Fæces, normal flora of, 1987 *et seq.*
Famine fever. *See* Relapsing fever
Farcy of cattle, 387, 1276
Fat metabolism of bacteria, 58
Fatigue, effect of on resistance, 1202
Fats, effect of on antigenic stimulus, 1115
lipins and waxes in bacteria, 45, 409
synthesis of, by bacteria, 59
Febris quintana or wolhynica, 1851
Feline enteritis, 1963
Ferkeltyphus, bacillus of. *See Salmonella typhi-suis*
Fermentation, study of by Pasteur, 2
reactions in identification of bacteria, **361**
Ferret, distemper in, 1962
influenza in, 1656, 1659, 1660
Fibrinolysin in bacterial infection, 1012
produced by *Str. pyogenes*, 591
Fibromatosis, infectious, of rabbits, 1979
Field mice or voles in relation to leptospiral infections, 1834–1836
to tsutsugamushi fever, 1849
Fièvre boutonneuse, 1848
Fildes' medium for growth of *H. influenzæ*, **793**
Filters, bacterial, 949
collodion, 951
gradocol, 951, 955
Filtrable forms of bacteria, 35, 408
viruses. *See* Viruses, animal
Filtration, factors affecting, 949
use in obtaining pure cultures, 355, 910
Finkler-Prior's vibrio, 525
Fixation of bacteria in tissues, 1044, 1045

Flagella, bacterial, 30
Flagellar antigens. *See* Antigenic structure of bacteria
Flaming in disinfection, 113
Flavacin, 177
Flavine, disinfection by, 135, 1776
Flavoprotein, rôle of in bacterial oxidation, 50
Fleas, in ætiology of plague, 1628 *et seq.*
in relation to murine typhus fever, 1845
Flexner's bacillus. *See Shigella*
Flies in relation to anthrax, 1733
to dysentery, 1563
to enteric fever, 1543
to limberneck of chickens, 1622
to summer diarrhœa, 1586
Fluorescin, 508–510
Foals, pyæmia in, 1404
Food poisoning, **1592–1612.** *See also* Botulism
chemical, 1592
diagnosis of, 1608
due to spray-dried egg, 1598, 1602, 1604
to "virus" preparations, 1603
epidemiology of, 1593
general bacteriology of, 1595
"infection" type of, 1596
caused by eggs, 1598, 1599
caused by milk, 1602, 2049
mode of infection of food in, 1602
human carriers, 1603
infected animals, 1598–1600, 1602, 1603
rats and mice, 1600, 1603
Salmonella types in, 1597
symptomatology of, 1592
type of food causing, 1601
investigation of outbreaks of, 1608
prophylaxis of, 1610
"toxin" type of, 1593, 1604
due to staphylococci, 1605
to various organisms, 1606
Foot-and-mouth disease, **1903–1907.** *See also* Chapter 55
experimental reproduction of, 1904
immunity in, 1906
mechanical factors in relation to lesions of, 1242
relation of to vesicular exanthema of swine, 1908
Ring-Impfung in, 1907
virus of, 1905
Foot-rot of sheep, 481, 1787
Forage poisoning in horses, 880, 1620
Formaldehyde, effect of on bacteria, 149
hypersensitiveness to, 1155
Formol toxoid in diphtheria immunization, 1392
in tetanus immunization, 1765
Forssman antibody, anaphylactic shock in relation to, 1144
antigen and antibody, 1089
antigen in bacteria, 282
Fowls, bacillary white diarrhœa in chicks, 1555
blue comb of, 1970
botulism of, 1622
Brucella infection of, 1719
bumble-foot of, 1349
cholera of, 1646
Fowls, choleraic disease of, 522
coryza of, 1799
infectious bronchitis of, 1970
infectious laryngo-tracheitis of, 1969
leucæmia of, 1976
limberneck in, 880, 1622
Newcastle disease of, 1658, 1969
paralysis of, 1976
plague or pest of, 1968. *See also* Chapter 55
pox and roup of, 1896, 1970. *See also* Chapter 55
Rous sarcoma of, 1977
Salmonella infection of, 1599
tuberculosis in, 428, 1343, 1348
Foxes, encephalitis of, 1923
Freezing, effect of on bacteria, 112
Frei test, 1871
Freundlich isotherm, 218
Freundlich series, 129
Friedländer's bacillus. *See Bacterium*, Friedländer group of
Frogs, red leg disease of, 647
Fumigacin, 177
Fumigatin, 177
Fusiformis, **477–484**
group characteristics of, 477
growth requirements of, 478
in infections of man, 1497, 1498, 1786
in mouth, 479
fragilis, 477, **483**
furcosus, 477, **484**
fusiformis, 477, **482**
melaninogenicus, 361, 484
necrophorus, 477, **479**
in calf diphtheria, 481, 1787
in foot-rot of sheep, 481, 1787
in human infections, 481, 1786
in labial necrosis of rabbits, 481, 1788
ramosus, 477, **483**
serpens, 477, **483**

Gaertner's bacillus. *See Salmonella enteritidis*
Gall-bladder, infection of, 1497, 1522
Gall-stones, effect of in promoting infection, 1497
Gamma globulin in measles prophylaxis, 1952
Gaol fever. *See* Typhus fever, classical
Garnet method of standardizing disinfectants, 146
Gas gangrene, 882, 885, 887, **1770–1783**
active immunization against, 1777
chemotherapy of, 1776
hyaluronidase in, 1773, 1774
in animals, 1778
in man, 1770 *et seq.*
antisera to, 1775
preparation and standardization of, 1776
bacteriology of, 1770
diagnosis of, 1774
mode of development of, 1772
prophylaxis and treatment of, 1775
lecithinase in, 1774
reproduction of in animals, 1772
Gas ratio, in differentiation of coli-ærogenes group, 661 *et seq.*

Gaseous disinfectants, 149
requirements of bacteria, 69
Gastro-enteritis. *See* Enteritis of infancy *and* Food poisoning
Gastric juice, bactericidal action of, 1021, 1987
Geese, septicæmia of due to *Trep. anserinum*, 1808
Genera, bacterial, summarized descriptions of, 318–323
Generation index, 89
Generation time, 89
of different organisms, 92
Genetic factors in anaphylaxis and hypersensitiveness, 1159
in immunity, 973
to *Pasteurella* and *Salmonella*, 974, 975, 1254
to tubercle bacillus, 975, 1298
Genital tract, factors determining normal flora of, 1025, 1210, 1996
Genus, bacterial, 316
Gerbilles, plague in, 1637
German measles, 1952
Germicides, practical application of, 149. *See also* Disinfectants *and* Disinfection
Giardia lamblia, enteritis due to, 1588
Gigantic acid, 177
" Ginger " paralysis, 1592
Gioddu, 750
Glanders, **1408–1415**
and farcy, 1408
control of in horses, 1415
diagnosis of, 1411
epidemiology of in animals, 1408
in man, 1411
experimental reproduction of in animals, 488, 491
immunity in, 1414
latent, 1409
mallein test in, 1412
prophylaxis of, 1414
Glandular fever, 1791
Glässer's bacillus. *See Salmonella typhi-suis*
Gliotoxin, 177
Globulins, antiviral antibodies in relation to, 1240
serum, nature of, 242
molecular weight of, 243
Glossary of terms employed in describing bacteria, 364–372, **369**
Glycolipoid antigens, 280, 1008
Goats, actinomycosis of due to aerobic *Actinomyces*, 1276
Brucella infection of, 1695, 1697, **1706**
contagious agalactia of, 945, 1867
tuberculosis in, 424, 429, 1343
Gonococcin, **1459**
Gonococcus. *See Neisseria gonorrhœæ*
Gonorrhœa, **1454–1460**. *See also Neisseria gonorrhœæ*
chemotherapy in prevention and treatment of, 1459
diagnosis of, 1456
in adults, 1454
in children, 1455
prevention and treatment of, 1459
pyrotherapy in, 1460
Gonorrhœa, reproduction of in man, 1456
Gonotoxin, **549**, 1456
Gradocol membranes, 951, 955
Grahamella, 903, 905
Gramicidin, 174
Gram's stain, 38
in identification of bacteria, 359
Granules, Babes-Ernst, 23, 449, 532
Bollinger's, 1492
glycogen and starch, 23
metachromatic, 23, 532
Much, 407
Paschen, 959, 1887
volutin, 23
Granulobacillus butyricus. *See Clostridium welchii*
Granulobacter saccharobutyricum. *See Clostridium butyricum*
Granuloma inguinale, 1790
Granuloma venereum, 1790
Gravis type of *C. diphtheriæ*. See *Corynebacterium diphtheriæ*
Grigoroff's Bacillus C., 764
Group and specific phases of flagellar (H) antigens, 278
Growth and death of bacteria, **80–99**
Growth curve of bacteria, 82
decline phase, 83, 97–98
lag phase, 82, 83–88
logarithmic phase, 82, 88–94
stationary phase, 83, 94–97
Growth of bacteria
effect of oxygen on, 96
of pH on, 72
of temperature on, 72
without multiplication, 84, 2045
Guarnieri corpuscles, 1887
Guinea-pig, normal, antitoxin in blood of, 1084
Guinea-pigs, anaphylactic shock in, 1137
epizootic hepato-enteritis of, 1960
epizootic lymphadenitis of, 1495
infectious paralysis of, 1932
meningo-encephalitis of, 1925
pneumococcal pneumonia of, 1499
pseudotuberculosis of, 1649
salivary gland virus of, 1943
tuberculosis in, 425, 429, 1325

H agglutinins in diagnosis of enteric fever, 1529–1540
in relation to antityphoid inoculation, 1537
H antigens, 277, 283
H→O variation, 291
Hæmin as bacterial vitamin, 68
Hæmobartonella, 903
Hæmolysins, 224. *See also* Antigen-antibody reactions. *For hæmolysins produced by various bacteria see under bacterium in question*
Hæmolysins, natural, 1089
Hæmolysis, 193, 224. *See also* Antigen-antibody reactions
hot-cold type, 616
Hæmolytic streptococci. *See Streptococcus*, hæmolytic group of

Hæmophilus, 786–810
antigenic relations of, 799
broncho-pneumonia in relation to, 1666
cultural characters of, 790
experimental infections with, 804–805
fermentative reactions of, 796
in Koch-Weeks conjunctivitis, 1496
in endocarditis, 1515
in wound infection, 1502
morphology of, 787
variation of, 805
bronchisepticus, 494, 495, 787 *et seq.*, **809**
causing pneumonia in animals, 1686
in distemper, 1962
relation to Perez's bacillus, 495
toxins of, 802
canis, 791 *et seq.*, 807
ducreyi, 791, **807**
influenzæ, 786–807
antigenic structure of, 799
broncho-pneumonia in relation to, 1666
fermentation reactions of, 796
growth requirements of, 790
hæmolytic strains of, 795 *et seq.*, 807
in meningitis, 1450
in normal nasopharynx, 1993
in sinusitis, 1499
mucoid type of, 799
pathogenicity of, 802
phases of, 801
rôle of in epidemic influenza, 1656
in swine influenza, 1661
smooth type of, 794 *et seq.*, **789**
summarized description of, 806
type of growth of, 794
X and V factors in relation to growth of, 791
influenzæ-suis, 791 *et seq.*, 807
para-influenzæ, 791 *et seq.*, 807
parapertussis, 801, 809
pertussis, 787, 791 *et seq.*, 809
antigenic structure of, 800
in relation to immunization against whooping cough, 1664
in whooping cough, 1662
pathogenicity of, 802
summarized description of, 809
toxins of, 802
Hæmorrhagic septicæmia. *See* Pasteurellosis
Hail, bacteria in, 2014
Haptens, 258. *See under* Antigens
Haptophore group, 197
Hasamiyami, 1834
Haverhill fever, 386, 1277, 1278
Haverhillia multiformis, 1277
Hay bacillus. *See Bacillus subtilis*
Hay fever, 1153
Heart, congenital malformations of, in relation to bacterial endocarditis, 1515
Heart-water fever, 929, 1862
Heat. *See also* Temperature *and* Disinfection
effect of on subsequent multiplication of bacteria, 116
use of in obtaining pure cultures, 354
Hedgehogs, foot-and-mouth virus in, 1903
Helber chamber, 81
Helvolic acid, 177
Hepatitis, enzootic, 1959
Hepatitis, infectious necrotic, 1780
Hepatitis, infective, 1793
relation of to serum and arsenic jaundice, 1794–1796
Hepato-enteritis of guinea-pigs, 1960
Herd infection and herd immunity, **1245–1266**
Heredity of immunity, 973 *et seq.*
in tuberculosis, 1298
d'Herelle's phenomenon. *See* Bacteriophage
Herpes febrilis, 1897. *See also* Chapter 55
encephalitis in, 1898
keratitis in, 1897, 1899
relation of to B and pseudo-rabies viruses, 1921
to encephalitis lethargica, 1916
Herpes genitalis, 1900
Herpes zoster, 1900
relation of varicella to, 1901
Heterologous, meaning of in relation to antigen-antibody reactions, 274
Heterophile antigen and antibody, 1089
antigen in bacteria, 282
Heterotrophic bacteria, 61
von Hibler's bacillus IX. *See Clostridium tertium*
Hiccup, epidemic, 1915
Hirschfeld's bacillus, 731. *See Salmonella paratyphi C.*
Hirst test, 1658, 1969
Hiss-Y bacillus, 689
Histaminase, relation of to anaphylactic shock, 1150
Histamine, action of on skin, 1149
antisera to, inhibition of, anaphylactic shock by, 1150
as kataphylactic agent, 1208
production of by tissues, 1149
shock, 1144
anaphylactic and anaphylactoid shock, relation of to, 1149
Histiocytes in relation to antibody formation, 1103–1106
to local immunity, 1185
phagocytosis of bacteria by, 1035 *et seq.*
Historical outline of bacteriology, **1–15**
Hodgkin's disease, 1981
Hofmann's bacillus. *See Corynebacterium*
Hofmeister series, 129
in relation to inhibition of agglutination, 214
inactivation of complement, 230
Hog cholera, 1963
Homologous, meaning of in relation to antigen-antibody reactions, 274
Hormones, relation of to immunity, 1210
Horses, actinomycosis due to aerobic *Actinomyces*, 1276
African sickness of, 1965
botriomycosis in, 1492
botulism in, 880, 1620
Brucella infection of, 1718
contagious pustular stomatitis in, 1895
epizootic abortion of, 1968
forage poisoning in, 880, 1620
grass disease or sickness of, 1621

Horses, infectious anæmia of, 1837, 1966
influenza in, 1662
joint ill of foals, 676
normal, diphtheria antitoxin in blood of, 1084
staphylococcal antitoxin in blood of, 1084
periodic ophthalmia of, 1943
strangles of, 1496
swamp fever of, 1966
tetanus of, 1767
tuberculosis in, 427, 429, 1342
ulcerative lymphangitis of, 1403
vesicular stomatitis of, 1907
Hot-cold lysis, 616
Hoyle's medium, 451, 1374
Humidity, effect of on resistance, 1203
Hyaluronidase formation, 591, 593
in bacterial infections, 1013
produced by various bacteria, 1013
protective action of, 1072
Hyaluronic acid in bacterial capsules, 1072
in tissues, 1013
Hydrocyanic acid, production of by *Ps. pyocyanea*, 510
Hydrogen peroxide, production of by bacteria, 72
toxic action of on bacteria, 72
Hydrogen sulphide, methods of examination for, 369
Hydrogen-ion concentration, 72. *See also* pH
effect of on disinfection, 114, 118
Hydrogenomonas flava, 505
pantotropha, 505
vitrea, 505
Hydrogen-transport in bacterial metabolism, 47
Hydrophile state in relation to antigen-antibody reactions 209
Hydrophobe state in relation to antigen-antibody reactions, 209
Hyperemesis hiemis, 1798
Hypersensitiveness, **1152–1160.** *See also* Allergy *and* Anaphylaxis
active and passive sensitization in man, 1153
bacterial, 1162
desensitization in, 1162–1164
relation of, to immunity, 1163
reaction of, inhibition by blocking antibody, 1159
desensitization in, 1160
genetic factors in, 1159
Prausnitz-Küstner reaction in, 1156

I.N.I. agent, 1875
Ice, bacteria in, 2014
Ice-cream, 1542, 1565, 1604, 2049
Ichthyosismus. *See* Botulism
Ictero-hæmoglobinuria of cattle, 1621
Identification of bacteria, 357–363
Idiosyncrasies, the, 1153
Immunity. *See also* Antibodies, Antigens, Antigen-antibody reactions *and under individual diseases*
acquired, 976
Immunity, active, 973
in experimental epidemics, 1252–1260
induction of by particular antigens, 1055
alcohol, effect of on, 1209
allergy in relation to, 1160, 1330–1336
antibacterial, **1034–1065**
antibodies, relation of to, 1051
different, relation of to, 1054
antigenic specificity of, 1054
antipneumococcal in rabbit, 1037, 1047
synthetic antigens in induction of, 1054
bactericidal and bacteriolytic reactions in relation to, 1046
efficacy of, 1057
histiocytes in relation to, 1035
leucocytes in relation to, 1036
macrophages in relation to, 1034
microphages in relation to, 1039
natural acquirement of, 1062
passive transference of, 1048
reactions of immunized as compared with normal animals in, 1047
reticulo-endothelial system in relation to, 1034 *et seq.*
antitoxic, 1029–1033
antitoxin in, 1030
avidity of, 1030
time of administration, importance of, 1031
endotoxins, relation of to, 1032
in relation to invasive infections, 1031
antiviral, active, 1229
cellular and humoral factors in, 1234
mechanisms of, 1232
passive, 1231
bacteriophage in relation to, 1215
benzene, effect of on, 1209
calcium as kataphylactic agent in relation to, 1206
cellular, 1053
in relation to local, 1187
congenital, 973, 1077
placenta, relation of to, 1077
diet, effect of on, **1190–1202**
enhancement of by non-specific substances, 1214
fatigue, effect of on, 1202
genetic, 973, 1078, 1079, 1298
genetic factors in, 1254
in experimental epidemics, 1254
grades of, 976
herd, **1245–1266**
hormones effect of on, 1210
humidity, effect of on, 1202
inanition, effect of on, 1201
infection, existing as result of, 1165
infection-, 1165–1166. *See also* Infection-immunity
innate, 973
local, **1180–1188**
non-specific factors in, 1185
measurement of, **981–994**
statistical methods, use of in, 983–1001

Immunity, mechanisms that hinder access of bacteria to tissues, **1019–1027**
effect of A-avitaminosis on, 1191
non-specific factors in, **1173–1178**
partial, 977
passive, 973
in relation to antibacterial immunity, 1048
to antitoxic immunity, 1029
penicillin, relation of to, 184
persistence of antigen in body in relation to, 1129
relation of to anaphylaxis, 1146
response to chilling as test of, 1203
seasonal variations in, 1205
Shwartzman phenomenon, relation of to, 1177
starvation, effect of on, 1193, 1201
sulphonamide drugs, relation of to, 1211
temperature, external, effect of on, 1202
tissue, in relation to local, 1187
ultra-violet rays, effect of on, 1204
various types of, **971–978**
virus diseases, in relation to, **1225–1242**
vitamins, effect of on, **1190–1199**
Immunization, active, 1257. *See also under individual diseases*
in experimental epidemics, 1257
relation of different antigens to, 1055
resulting from infection, 1062
combined active and passive, 1117, 1401
concentrated, 1115
natural, in experimental epidemics, 1254
side-to-side inoculation in, 1231
Impaction paralysis of cattle, 1621
Impetigo contagiosa, 1493
Impressed variations in bacteria, 290, 291
Inanition, effect of on resistance, 1201
Inclusion blennorrhœa, 1880
Inclusion bodies, 958, 1228. *See also* Bollinger inclusion bodies, Borrel bodies, Guarnieri corpuscles *and* Paschen granules
Indicator media, use of in obtaining pure cultures, 356
Indole, methods of examipation for, 367
Infantile paralysis. *See* Poliomyelitis
Infants, enteritis of. *See* Enteritis of Infancy
Infection, bacterial, 1002 *et seq.*
by the mouth, experimental, 1045
cellular reactions in, 1007
mechanisms of, **1002–1016**
mechanisms that hinder access of bacteria to tissues, **1019–1027**
of wounds, 1500–1503
sequence of immunological events in, 1062
herd, **1245–1267**
latent, herd infection in relation to, 1246
Infection-immunity, 1165, 1813
Infectivity, bacterial, relation of to virulence, 1260
Influenza, **1655–1662**
active immunization against, 1659
antiviral sera in, 1660
bacillus. *See Hæmophilus influenzæ*
diagnosis of, 1659
Hirst test in, 1658
Influenza in ferret, 1656, 1659, 1660
in swine and other animals, 1661–1662
relation of human to swine virus in, 1656 1657
rôle of *H. influenzæ* in, 1656
virus of, 1656–1659
Influenzal meningitis, 1450
Insect vectors of infection. *See under* Fleas, Flies, Lice, Mosquitoes, Ticks *and individual diseases*
Interference phenomenon, 1236
Intermedius type of *C. diphtheriæ*. *See Corynebacterium diphtheriæ*
of coli-ærogenes, 663, 2024
Intestinal bacteria
as food-stuffs, 1991
destruction of vitamins by, 1992
effect of sulphonamides on, 1991
synthesis of vitamins by, 1991
Intestinal obstruction, clostridial infections in, 1777
Intestinal toxæmia, 1990
Intestines, bactericidal mechanisms in, 1021, 1988
importance of micro-organisms in, 1989
local immunity of, 1183
normal flora of, 1986–1992
Involution forms of bacteria, 33
Irradiation, effect of on resistance, 1203
Isoagglutinins, 1089
Isolation, effect of on incidence of infective disease, 1265
Isolysins, 1089

Jaeger's coccus, 531, 532, 553
" Jake " paralysis, 1592
Japanese, or B, encephalitis, 1917
Japanese River fever, 1849
Jaundice, arsenic, 1795
catarrhal, 1794
epidemic, 1793
infective, 1793
leptospiral in animals, 1837
in man, 1828–1837
serum, 1794
Joest-Degen corpuscles, 1922
Johne's bacillus. *See Mycobacterium johnei*
Johne's disease, 441, **1363**
bacteriology of, 441
johnin reaction in, 1365
tuberculin reaction in, 1365
Johnin, 1365
Joint ill in foals, bacillus associated with, 676
in lambs, 1284
streptococcal, of lambs, 1496
Joints, pyogenic infections of, 1496. *See also* Arthritis
Jones and Handley's medium for inhibiting *Proteus*, 1528

K.H. reaction in diagnosis of glanders, 1414
Kahn test, 1819
interpretation of, 1820 *et seq.*
Kataphylaxis, 1206, 1750, 1773
Kauffmann-White schema, 712–716
Kedani, 1849
Keel disease of ducks, 1599

Kefir, 750
Kenya fever, 1848
Keratitis herpetica, 1899
Keratoconjunctivitis, epidemic, 1902
Kidney, pulpy disease of lambs, 1782
  pyogenic infections of, 1499
Kikuyu, diet and resistance of, 1200
Kissélo-mléko, 750, 763, 764
Klebsiella, 654, 655
Koch, 9 *et seq.*
Koch's phenomenon as example of allergy, 1161, 1326
Koch's postulates, 1002
Koch-Weeks bacillus, 791 *et seq.*, 807
  in conjunctivitis, 1496
Kupffer cells, phagocytic action of, 1035 *et seq.*

LD50 dose of lethal substances, 995
Lf and Lr dose of toxin, 1384
Lo and L + dose of toxin, 239, 1384
Labial necrosis of rabbits, 481, **1788**
Laboratory infections
  in cholera, 1420
  in dysentery, 1571
  in glanders, 1411
  in louping-ill, 1925
  in lymphocytic choriomeningitis, 1933
  in psittacosis, 1872
  in scarlet-fever, 1464
  in tularæmia, 1721
  in typhus fever, 928, 1846
  in undulant fever, 1701, 1702
Lactiformans, 756
Lactobacillus, **750–765**
  antigenic structure of, 754
  biochemical reactions of, 753
  classification of, 755
  metabolism of, 752
  methods of isolation of from natural sources, 752
  pathogenicity of, 754
  resistance of, 752
  acidophil-ærogenes, 753, 758, 761. *See also L. brevis*
  acidophilus, 750 *et seq.*, 757, 1987, 1991, 1996
  berolinensis, 756
  bifidus, 750 *et seq.*, 762, 763, 1987
  brevis, 756, 758, **761**
  buchneri, 756
  bulgaricus, 750 *et seq.*, 763, 1990
  casei, 756, 757
  caucasicus, 750, 757, 763
  exilis, 750, 757, 764
  fermenti, 756
  odontolyticus I and *II*, 753 *et seq.*, 760, 761
  pastorianus, 756
  pentoaceticus, 753. *See also L. brevis*
  plantarum, 757
  wehmeri, 756
Lag phase of growth, 83
Lakes, bacteria in, 2015
Lambs. *See* Sheep
Lamziekte, 880, 1621
Laryngo-tracheitis of fowls, 1969
Latent infection. *See under individual diseases*
Latent infection, in relation to herd infection, 1246
Lattice hypothesis of antigen-antibody reactions, 208, 220
Leben raib, 750
Lecithinase in bacterial infection, 867, 1774
Leeuwenhoek, van, 2
Leifson's desoxycholate citrate medium, 1528
  sodium selenite medium, 1528
Lemming fever, 1721
Lemon's formula, 90
Lepromin, 1361
Leprosy, **1358–1363**
  bacillus. *See Mycobacterium lepræ*
  bacteriology of, 434–440
  due to rat leprosy bacillus, 1359
  in rats. *See* Rats
  Wassermann reaction in, 1360
Leptospira, 908 *et seq.*, 919–924
  in water, 1829–1831
  infections due to, 1828–1838
  tabulated diseases due to, 1835
  akiyami, 923, 1834, 1836
  Andaman A and B types, 924, 1834, 1836
  australis A and *B*, 923, 1836
  autumnalis, 923, 1834, 1836
  bataviæ, 923, 1834
  biflexa, 921, 924, 1831
  canicola, 924, 1837
  grippo-typhosa, 923, 1836
  hebdomadis, 923, 1836
  icterohæmorrhagiæ, 911, 912, 919
    in canine jaundice, 1837
    in infection of rats, 1830
    in Weil's disease, 1828
  javanica, 924
  mitis, 923
  oryzeti, 924
  pomona, 923, 1837
  Rachmat type, 923, 1834
  Salinem type, 923, 1834
  sejroe, 924, 1836
  Swart van Tienen type, 923, 1834
Leucæmia of fowls, 1976
Leucocidin, pneumococcal, 592
  staphylococcal, 616
  streptococcal, 590
Leucocytes, in relation to immunity, 1036
  killing of bacteria by without phagocytosis, 1175
    in antiviral immunity, 1233
  rôle of in immunity, 1174
  variation in phagocytic power of, 1174
Leuconostoc citrovorus, 628
  dextranicus, 628
  mesenterioides, 627
Leukins, 1173
Leukotaxine, 1044
Levinthal's medium for growth of *H. influenzæ*, 793
Lice in relation to epidemic typhus fever, 1841–1844
  to infectious anæmia of rats, 1798
  to relapsing fever, 1805
  to trench fever, 1851
Life-cycle of bacteria, 34, 87
Light, effect of on bacteria, 102

Limberneck in chickens, 880, 1622
Lindner's initial bodies in trachoma, 1878
Lipopolysaccharide antigens. *See* Glycolipoid antigens
Liquid air, effect of on bacteria, 112
Lister, 11
Listerella, 395–403, **401**. *See Erysipelothrix monocytogenes*
Lister's method of counting bacteria, 81
Litmus milk, changes produced by bacteria in, 367
Liver flukes in causation of black disease, 1781
Local anaphylactic reactions, 1151
Local formation of antibodies, 1101
Local immunity, **1180–1188**. *See also* Immunity, local
Localization of bacteria, 1005, 1045
Loewenberg's bacillus, 494, 667, 1789
Logarithmic phase of growth, 82, 88–94
Lombriz of sheep, 1646
Lone Star fever, 1846
Louping-ill, 1925
Lübeck catastrophe, 1306, 1341
Lukes, canine typhus of, 1837
Lungs, relative sterility of normal, 1024
Lygranum, 1871
Lymph capillaries, permeability to foreign matter of, 1042
Lymph glands. *See Lymph nodes*
Lymph nodes, fate of bacteria in, 1043
  fate of viruses in, 1045
  filtering action of, 1043
  formation of antibodies in, 1104
Lymphadenitis, epizootic, of guinea-pigs, 1495
  streptococcal, of dogs, 1496
Lymphadenoma, 1981
Lymphatic blockade, 1044
  system, availability of antibody in, 1061
Lymphocytic choriomeningitis, 1932
Lymphogranuloma inguinale, 1869
Lymphopathia venereum, 1869
Lysis. *See* Bacteriolysis, Hæmolysis *and* Bacteriophage
Lysozyme, **1020**, 1022, 1175
  in tears, 1025
  in tissues, 1998

MacConkey's medium in cultures from enteric fever, 1527
  in water analysis, 2022
McLeod's medium, 451, 1374
Macrophages, 1035. *See also* Histiocytes
Mad itch, 1941
Madura disease, 1275
Malignant catarrh of cattle, 1966
  œdema, 1778
  pustule, 1733
Mallein test, 1412
Malta fever, 1693–1697. *See also* Undulant fever, due to *Br. melitensis*
Maltase in serum, 367, 534
Manchester bacillus. *See Shigella newcastle*
Marfanil, 158, 167
Marseille, exanthematic fever of, 1848
Masai, diet and resistance of, 1200
Mass law, in antigen-antibody reactions, 219
  in enzyme adaptation, 293
Massauah vibrio, 526
Massol's bacillus. *See Lactobacillus bulgaricus*
Mastitis, suppurative in nursing mothers, 1492
Mastitis in cattle, 1493
  C. pyogenes associated with, 1494
  staphylococci associated with, 1493, 1494
  streptococci associated with, 581, 1493, 1494
Mazun, 750
M-concentration of living organisms in culture, 94
Measles, 1949–1952. *See also* Chapter 55
  latent infection in, 1226
  prophylaxis of, 1950–1952
  reproduction of in animals, 1949
Meconium, bacteria in, 1989
Media indicator, use of in obtaining pure cultures, 356
  selective, use of in obtaining pure culture, 355
  solid, introduction of by Koch, 10
Mediterranean fever. *See* Undulant fever
Melioidosis, 1415
Melitin reaction in diagnosis of undulant fever, 1705
Membrane, cytoplasmic, of bacterial cell, 27
Meningitis, **1431–1451**
  acute, lymphocytic type of, 1932
  aseptic, 1932
  cerebrospinal, 1431–1449. *See also Neisseria meningitidis*
    carrier epidemic in, 1434
    carrier rate in, 1434, 1442
      effect of overcrowding on, 1434–1436
    chemoprophylaxis in, 1443
    chemotherapy in, 1448
    diagnosis of, 1438
      by examination of petechial skin lesions, 1441
    epidemiology of, 1432–1438
    mode of spread of, 1433
    prophylaxis of, 1441
    rhinopharyngitis in, 1434
    routes of infection in, 1436
      direct extension from nose, 1436
      hæmatogenous, 1437
    serum treatment of, 1444–1448
    vaccination in, 1444
  influenzal, 1450
  in Weil's disease, 1829, 1837
  pneumococcal, 1449
  streptococcal, 1449
  tuberculous, 1290, 1449
  uncommon forms of, 1451
Meningococcus. *See Neisseria meningitidis*
Meningo-encephalitis of rabbits, 1916, 1924
Meningo-pneumonitis virus, 1876
Metabolic properties of bacteria, 360, 367
Metabolism of bacteria, 42–74
  mechanisms of, 46 *et seq.*
Metachromatic granules, 23, 449, 532
Metallic salts, effect of in antibody formation, 1115, 1122
Metals, effect of on bacteria, 120–130

Methanomonas methanica, 505
Methods of examination of bacteria, 364–369
Methyl red test, 368, 662
Methylene blue, use of in study of oxidation-reduction reactions, 50
Methylene blue reduction, methods of examination for, 369
test in examination of milk, 2045
Mexican typhus, 1841, 1845
Mice, anaphylaxis in, 1139
choriomeningitis in, 1933
ectromelia of, 1278, 1909
in experimental epidemics, 1253–1264
epidemic in, due to Pasteurella-like organism, 779
field. *See* Field mice
in relation to food poisoning, 1600
infectious catarrh of, 1799
infective arthritis of, 386, 1278
influenza of, 1799
pasteurellosis of, 777, 1254
plague in, 1637
pseudotuberculosis of, 461, 1649
tuberculosis in, 426, 429
typhoid in. *See* Mouse typhoid
Michaelis's acid agglutination test, 692
Micrococcus, **623–625**
ærogenes, 625
agilis, 627
ascoformans, 1492
buccalis, 625
caseolyticus, 624
catarrhalis. *See Neisseria*
conglomeratus, 623
coronatus, 624
flavus, 623
freudenreichii, 624
gingivalis, 625
luteus, 623
melitensis. *See Brucella*
minimus, 625
pharyngis cinereus. *See Neisseria*
pharyngis flavus, i, ii and iii. *See Neisseria*
pharyngis siccus. See *Neisseria*
prodigiosus. *See Chromobacterium*
radiatus, 624
tetragenus, 607, **624**
ureæ, 624
varians, 623
Microphages, 1039. *See also* Polymorphonuclear leucocytes
Micromonospora, 382
Microscopy, electron, 18
in study of filtrable viruses, 952
limitations of, 18, 951–953
Midland cattle disease, 1621
Mid-piece of complement, 229
Milk, bacterial flora of, 2036–2039
pathogenic bacteria in, 2038
Actinomyces muris, 1278
anthrax bacilli, 1735
Brucella abortus, 1700, 1713, 2038
Brucella melitensis, 1695, 1697, 1707, 1711
Brucella suis, 1702, 1711
Milk, bacterial flora of, pathogenic bacteria in, *Corynebacterium diphtheriæ*, 1390, 2039
foot-and-mouth virus, 1905
Staphylococcus aureus, 1494, 1605, 2039
streptococci, 1471, 1494, 2039
tubercle bacilli, 1317, 1344, 2038
bacteriological grading of, 2041–2049
cleanliness, safety, keeping quality and pasteurizability of, 2039
designations of, 2048
diseases borne by, 2049. *See also* individual diseases
diarrhœa and dysentery, 1565, 1588, 1590, 2049, 2050
diphtheria, 1390, 2049, 2050
enteric fever, 1542, 2049, 2050
food poisoning, 1602, 1605, 2039
Haverhill fever, 1278
poliomyelitis, 1927
rat-bite fever, 1278
scarlet fever and other streptococcal infections, 1471, 2039, 2049, 2050
tonsillitis, 1478, 2049, 2050
tuberculosis, 1289, 1317, 1336, 2049
undulant fever, 1695, 1697, 1700, 2049
dried, as dietary factor in relation to immunity, 1200
foot-and-mouth virus in, 1905
grades of
accredited, tuberculin tested, pasteurized, heat-treated, 2047–2049
sterilized, 2053
litmus, changes produced by bacteria in, 367
pasteurization of, 1318, 1319, 1336, 1588, 2048, **2051**
production of clean milk, 2037
of safe milk, 2050–2053
Milk-borne disease. *See* Milk, diseases borne by
"Miniature" scarlet fever, 1465
Mineral salts, possible effect of on resistance, 1201
waters, bacteria in, 2015
Minimal Hæmolytic Dose (M.H.D.) of complement, 227
of hæmolysin, 227
Minimal Lethal Dose (M.L.D.) of toxin, 238
of diphtheria toxin, 1384
Miscellaneous bacteria, group of, 898–905
Mist bacillus. *See Mycobacterium stercoris*
Mite fever, 936, 1849
Mitis type of *C. diphtheriæ*. *See Corynebacterium diphtheriæ*
Mitogenetic rays, 98
Moeller's grass bacilli i and ii, 404
Moloney reaction, 1393, 1396
Molluscum contagiosum, 1978
Mongoose, in relation to rabies, 1936
to virus pneumonia, 1876
Mongoose virus, 1876
Monkeys, anaphylaxis in, 1139
experimental pneumococcal pneumonia of, 1668
follicular conjunctivitis of, 1878
tuberculosis in, 427, 429, 1343

Monomolecular reaction, similarity of disinfection to, 137–140
Mononucleosis, infectious of man, 1791
of rabbits, 1286
Morax-Axenfeld bacillus, 898
Moraxella, 899
Morgan's bacillus. *See Proteus morgani*
Morphology of bacteria, 16–39
in identification, 358
variations in, 290
Morvine, 1414
Moscow typhus, 1845
Mosquitoes in spread of dengue fever, 1958
of yellow fever, 1953
Motility, use of in obtaining pure cultures, 353
Mould products, miscellaneous, 179
Mouse pneumonia virus, 1876
Mouse septicæmia, bacillus of, 395, 1286
Mouse typhoid, experimental epidemics of, 1253 *et seq.*
Mouth, removal of bacteria from, 1020
Much granules, 407
Mucin, effect of on bacterial infection, 1209
Mucus, rôle of in removal of bacteria, 1022
Mud fever. *See* Swamp fever
Muller's tetrathionate medium, 1528
Mumps, 1952
Mutation, bacterial, 295. *See also* Variation of bacteria
Myalgia, epidemic, 1960
Mycetoma, 387. *See also* Madura disease
Mycobacterium, **404–442**
acid-fast bacteria, 404
antigenic structure of, 422
avian types of, 404
biochemical reactions of, 422
chemical structure of, 409
classification of, 431
cold-blooded types of, 404
cultural reactions of, 411
habitat of, 405
mammalian types of, 404, **431**
metabolism of, 419
morphology of, 405
of voles. *See Mycobacterium muris*
optimum hydrogen-ion concentration for growth of, 419
optimum temperature for growth of, 420
oxygen requirements of, 420
pathogenicity of, 423
resistance of, 417
rough and smooth forms of, 416
saprophytic types of, 404, 414
staining of, 405
tabulated reactions of, 435–437
toxin production by, 422
variation of, 415
butyricum, 404
johnei, 404, **441**
lepræ, 404, **434**
muris, 405–406, 411, 413, 420, 423–429, **432, 435–437**
of rat leprosy, 404, **440**
phlei i and ii, 404
smegmatis, 405
stercoris, 405
Mycobacterium tuberculosis, 404 *et seq.*, **433**
aberrant types of, 431
attenuated types of, 431
avian and cold-blooded types of, cultural differentiation of, 414
bovine type of, 404
cellular reactions to products of, 410,
chemical structure of, 409
differential characteristics of, tabulated, 432, 435–437
dysgonic type of, 412, 431
effect of glycerine on growth of, 420
eugonic type of, 412, 431
filtrable forms of, 408
human and avian types, differentiation of, by pathogenicity, 430
culturally, 413
serologically, 422
human and bovine types of, differentiation of, by pathogenicity, 430
culturally, 412, 420
human type of, 404
in suppurative lesions of the urinary tract, 1500
lesions caused by dead bacilli, 431
minimal infecting dose of for guinea-pigs, 425
Much granules of, 407
murine type of, differentiation of, 413, 435–437
pathogenicity of, 423–428
pigment formation by, 413, 414, **421**
summarized description of, 433
supposed life-cycle of, 408
tuberculin formation by, 410, **1327**
Yersin type of disease caused by, 426
Myelitis, due to B virus, 1921
due to herpes virus, 1921
due to rabies virus, 1921, 1935
Mykol, 407
Myositis, clostridial, 1771. *See* Gas gangrene
Myxomatosis, infectious, of rabbits, 1978

Nairobi disease of sheep and goats, 1967
Nanukayami, 1834
Nasal mucosa, effect of temperature and humidity on, 1203
Nasik vibrio, 516, **526**
Nasopharynx, normal flora of, 1992 *et seq.*
Nastin in treatment of leprosy, 1361
Native populations, Schick and Dick reactions among, 1080–1082
tuberculosis in, 1296
Natural antibodies. *See* Antibodies, natural
Neapolitan fever. *See* Undulant fever due to *Br. melitensis*
Necrobacillosis, 1786
in animals, 1787
in man, 1786, 1788
Necrosin, 1044
Necrosis bacillus, 479. *See also Fusiformis necrophorus*
Necrotuberculosis, 431
Negative phase in antibody production, 1116
Negri corpuscles, 1937
Neill's medium, 451, 1374

Neisseria, **531–556**
antigenic structure of, 535, 538, 545
biochemical reactions of, 534
maltase in serum, 534
chemical fractionation of, 535, 539, 547
classification of, 536
growth requirements of, 532
pathogenicity of, 535, 539, 548
pigment formation by, 534, 536, 556
resistance of, 534
tabulated reactions of, 554, 555
variation in, 534, 536, 537, 544
catarrhalis, 531 *et seq.*, **551**
flavescens, 531, **556**
gonorrhœæ, 531 *et seq.*, **543–550**
differentiation of from *N. meningitidis*, 550
disease caused by, 1454–1460
in suppurative lesions of the urinary tract, 1500
media for cultivation of, 543
toxin production by, 549
meningitidis, 531 *et seq.*, **537–543**
autolysin of, 538
differentiation of from *N. gonorrhœæ*, 550
disease caused by, 1431–1449
toxin production by, 541
pharyngis, 535, 537, 553
pharyngis cinerea, 531, 553
pharyngis flava i, ii and *iii*, 531 *et seq.*, 552
pharyngis sicca, 531 *et seq.*, 553
Neisser-Wechsberg phenomenon, 228
Neonatal diarrhœa, 1583
Newcastle bacillus, 690. *See Shigella*
Newcastle disease of fowls, 1658, 1969
Niacin. *See* Nicotinic acid
Nicotinic acid as bacterial vitamin, 67, 163
Nicotinamide, 165
Nitrate reduction, methods of examination for, 368
Nitrates, effect of on anaerobic growth, 54
Nitrobacter, 504
winogradskyi, 504
Nitrogen-fixing bacteria, 497
Nitrogenous constituents of bacteria, 43
metabolism of bacteria, 55
Nitrosococcus americanus, 503
Nitrosomonas, 502–504
europœa, 503
Nocardia, 374, 382
Nocard's bacillus, 1873
Noma, 1810
Nomenclature of bacteria, 310–323
Normal curve, 985
Normal flora of body, **1984–1999**
alimentary tract, 1986–1992. *See also* Intestines
factors affecting, 1988
blood and internal organs, 1998
conjunctiva, 1998
factors affecting, 1984–1985
mouth, 1986
respiratory tract, 1992–1996
persistence of species in, 1995
skin, 1997
technique of examination, 1984
Normal flora of body, tonsils, 1992
urethra, 1997
vagina and vulva, 1996
Nose, arrest of bacteria in, 1023
normal flora of, 1992 *et seq.*
Notatin, 178
Nuclear apparatus of bacteria, 19
as genetical postulate, 289
Nucleic acid, effect of on antibody formation, 1123
Nuclein, increase of opsonic power following injection of, 1175
Null hypothesis, in statistical reasoning, 1000
Nutritional requirements of bacteria, 61
and essential nutrients, 63
and evolutionary series, 62
and growth stimulants, 63
as basis of classification, 312

Oagglutinins in diagnosis of enteric fever, 1529–1540
in relation to antityphoid inoculation, 1537
O antigens, 277, 283
Ohara's disease. *See* Tularæmia
Oils, animal, effect of on bacteria, 137
essential, effect of on bacteria, 136
Omentum, rôle of in peritoneal infections, 1040
Oosporosis, 1270
Opacity of a bacterial suspension, factors determining, 81, 85
Ophthalmia neonatorum, 1455, 1460
Ophthalmia, periodic, of horses, 1943
Opsonins, 194, 235. *See also* Antigen-antibody reactions
increase of, induced by injection of nuclein, 1175
non-specific, 235, 237
Optimal antigen-antibody ratio in agglutination, 212
in precipitin reaction, 201 *et seq.*
with constant antibody, 202, 204
with constant antigen, 202, 204
relation of to chemical equivalence, 204
of constant-antigen ratio to constant-antibody ratio, 202, 209
Optimal proportions in agglutination, 213
in complement fixation, 233
in precipitation, 201
Oral sepsis in relation to endocarditis, 1515
Organic acids as disinfectants, 119
as substrates for fermentation tests, 706
Ornithosis, 1874
Oroya fever, 903, 1796
Osmotic effect of salts on bacteria, 127
Osteomyelitis, 1496, 1506
Osteophagia, 1621
Otitis media in rats, 1499
Owls, virus disease of, 1971
Oxalates in metabolism of *Pf. whitmori*, 493
Oxalates, production of by *Pf. whitmori*, 492–493
Oxidase, bacterial, 48
Oxidation-reduction potential, measurement of, 50
reactions in bacterial metabolism, 50, 71

Oxygen, effect of on growth of bacteria, 96
on survival of bacteria, 2017
variation in requirement of, by bacteria, 360
Oxygen lack, effect of on antibody formation, 1122
Oysters, 2031
Ozæna, 494, 1788
Abel-Loewenberg bacillus in, 667 *et seq.*
bacillus of Perez in, 494
Ozone, effect of on bacteria, 149

Pahvant Valley fever. *See* Tularæmia
Pantothenic acid as bacterial vitamin, 67, 164
Pantoyltaurine, 164
Papillomatosis of rabbits, infectious, 1980
oral, 1980
Paracholera vibrios, 520
Para-aminobenzene sulphonamide. *See* Sulphanilamide
Para-aminobenzoic acid, as bacterial vitamin, 162
as antagonizer of sulphonamide compounds, 161
Paracolon bacilli, 676, 715
Salmonella antigens in, 715, 744
Vi antigen in, 715, 744
Para-influenza bacilli, 791 *et seq.*
Paralysis, bulbar due to pseudorabies virus, 1941
infectious, of guinea-pigs, 1932
Parameningococcus, 538
Para-Shiga bacilli. *See Shigella*
Paratyphoid fever. *See* Enteric fever
Parotitis, epidemic, 1952
Parrots, psittacosis in, 1872
tuberculosis in, 427, 429, 1343
Paschen granules, 959, 1887
Passive anaphylaxis, 1140
immunity. *See* Immunity, passive
Pasteur, 2 *et seq.*
Pasteurella, **767–784**
antigenic structure of, 773
biochemical reactions of, 772
classification and identification of, 778
colonial variants of, 769
cultural characteristics of, 769
differential characteristics of, tabulated, 779
hæmolytic group of, 778
in experimental epidemics, 1252–1259
metabolism of, 771
organisms resembling, in acute contagious pneumonia of sheep, 779
in epidemic in mice, 779
pathogenicity of, 775
resistance of, 770
Salmonella antigens in, 703, 773
variations in virulence of, 775
Vi antigens in, 774
aviseptica, 767 *et seq.*
boviseptica, 767
bubalıseptica, 772
lepiseptica, 767, 770, 771
murıseptica, 767
oviseptica, 767
Pasteurella pestis, 767 *et seq.*, **780**
disease caused by, 775, 1627–1646
pseudotuberculosis, 767 *et seq.*, **782**
disease caused by, 777, 1649
motility of, 768
septica, 767 *et seq.*, 778, 781
diseases caused by, 777, 1646–1649
suiseptica, 767
vitulıseptica, 767
Pasteurellosis, **1646–1649**
experimental epidemics of in mice, 1254
experimental reproduction of in animals, 777
of rats, 1639
Pasteurization of milk, 1318, 1319, 1336, 1588, 2048, **2051**
Pathogenicity in identification of bacteria, 362
of various bacteria. *See under individual bacterium*
methods, use of in obtaining pure cultures, 356
Patulin, 177
Paul-Bunnell test, 1792
Paul's test in diagnosis of smallpox, 1888
Pemphigus neonatorum, 1492
Penatin, 178
Penfold's hypothesis of lag, 84
Penicidin, 178
Penicillia, 175
Penicillic acid, 178
Penicillin, 175, **179–185**
acquired resistance of bacteria to, 180
action of in animal body, 184
assay of, 181
discovery of, 175
laboratory use of, 182
mode of action of, 182
range of activity of, 175, 180
use of in obtaining pure cultures, 356, 1664
Penicillinase, 180
Peptone shock, 1144
possible relation of histamine to, 1151
Perez's bacillus, 494
Periostitis, 1496
Peritoneal cavity, experimental infections in, 1039
passage of bacteria from to blood stream, 1041
Peritonitis, 1498
clostridial infections in, 1777
Peroxidases in bacterial metabolism, 48
Pertussis. *See* Whooping Cough and *Hæmophilus*
Petit's bacillus, 899
Pfeifferella, **486–496**
antigenic structure of, 488
classification of, 490
group characters of, 486
pathogenicity of, 488
relation of to *Actinobacillus*, 490
mallei, 486 *et seq.*, **490**
disease caused by, 1408–1415
whitmori, 486 *et seq.*, **492**
disease caused by, 1415
oxalate production by, 492–493
Pfeiffer's reaction, 192, 1425, 1806
pH, effect of on bacterial growth, 72

hagocytes, 1039
possible protective action of on bacteria, 1060
hagocytosis, 1039. *See also* Opsonins, Bacteriotropins *and* Immunity, antibacterial
aggressins, inhibition of by, 1069
effect of protein deficiency on, 1201
electric charge in relation to, 235
genetically determined, 975
rôle of opsonins in, 193
variations in phagocytic power of leucocytes, 1174
helps' method of standardizing disinfectants, 148
henols, effect of on bacteria, 133
hlebitis, 1491
hlebotomus fever, 1959
'*hlebotomus noguchi* in relation to *verruga peruana*, 1797
hosphatase, production of by diphtheroid bacilli, 453
test for heated milk, 2051
hotodynamic sensitization, effect of on bacteria, 106
on bacteriophage, 107
hotomicrography, use of ultra-violet light in, 18, 952
hotosynthesis in bacteria, 61
igeon-pox, 1896
igment formation by bacteria, effect of salts on, 633, 635, 637
in identification of bacteria, 361
inhibition of by calcium, 635
variation in, 299
igments, bacterial, rôle of in metabolism, 73
in photosynthesis, 61
igs. *See* Swine
'lacenta, relation of to congenital immunity, 1077, 1092
'lague, 775, **1627–1646**
chemotherapy in, 1646
diagnosis of in man, 1641
in rats, 1631, 1638
thermoprecipitin test in, 1639, 1641
differential diagnosis of from tularæmia, 1722
differentiation of from pseudotuberculosis, 1640
epidemiology of, 1627
experimental reproduction of in animals, 775
fleas in ætiology of, 1628 *et seq.*
in animals, other than rats, 1637
in rats, 1628 *et seq.*
experimental transmission of, 775, 1631
increasing immunity of rats to, 1635, 1641
mode of spread of in Bombay, 1628, 1634
in Egypt and other parts, 1636–1638
pneumonic, 1638
prophylaxis of, 1642
serum treatment in, 1645
vaccination against, 1643
'lakins, 1173
Plate method of counting bacteria, 82
in milk, 2043
in water, 2021
use of in obtaining pure cultures, 352
Platelets, rôle of in clearing mechanism of blood stream, 1036
Pleurodynia, epidemic, 1960
Pleuropneumonia group of organisms, **939–948**
cultivation of, 940
diseases caused by, 945–948 1867
morphology of, 941–944
organism of contagious agalactia, 945, 1867
other characters of, 944–945
strains of, in dogs, 946
in guinea-pigs, 947
in man, 947
in mice, 947
in rats, 946
lettered and numbered types of, 946, 947
saprophytic pleuropneumonia-like, 947
of cattle, 1867
due to *Pasteurella*. 1647
of swine, 1964
Pneumobacillus. *See Bacterium*, Friedländer group of
Pneumococcal meningitis, 1449
polysaccharides. *See Streptococcus pneumoniæ*
septicæmia, experimental study of in rabbits, 1037, 1048
Pneumococcus, 561. *See Streptococcus pneumoniæ*
Pneumonia, **1668–1686**
active immunization against, 1683
aggressive action of haptens in, 1071, 1679
antipneumococcal serum, and sulphonamide drugs, combined treatment of with, 1682
from horses, 1675–1677
from rabbits, 1678
standardization of, 1679
treatment of with, in animals, 1669
in man, 1675–1679
atypical, 1876
bacteræmia in, 1670
chemotherapy of, 1681
contagious of sheep, 779, 1648
enzyme treatment of, 1682
experimental in animals, 1669
frequency of different pneumococcal types in, 1671 *et seq.*
in animals, due to *H. bronchisepticu* 1686
due to *Pasteurella*, 1646–1648
in guinea-pigs, 1499
in rats, 1499
infectious of goats, 1646
mortality of in relation to infecting type, 1671–1673
polysaccharides, excretion of in, 1678
relation of " carrier " types to, 1673 *et seq.*
as a contagious disease, 1674
skin reactions to pneumococcal polysaccharides, 1685

Pneumonia, Type III, treatment of with bacterial enzyme, 1682
typing of infecting pneumococcus in, 1678
vaccination against, 1683
vitamin A in relation to, 1195
viruses in, 1875
Pneumonic plague, 1638
Poisoning, food. *See* Food poisoning
ptomaine, 1595
Polar staining, 37
Poliomyelitis, 1926–1932. *See also* Chapter 55
latent infection in, 1226
natural antibodies to, 1226
Polysaccharide antigens of various bacteria, 279. *For particular bacteria see under organisms in question*
Polysaccharides bacterial, as aggressins, 1071, 1679
as haptens, 258
excretion of, in disease, 1678
in stimulation of antibody production, 1119, 1684
Porges' method for capsule removal, 671
Portal pyæmia, 1491
Potential difference in relation to agglutination, 214
to phagocytosis, 235
Poxes, animal, 1895–1897
Prausnitz-Küstner reaction in hypersensitiveness, 1156
Precipitin reaction, 200. *See also* Antigen-antibody reactions
Precipitins. *See under* Antigen-antibody reactions
formation of, 1109
protective action of, 1057
Preisz-Nocard bacillus. *See Corynebacterium ovis*
Pre-school child, immunization of against diphtheria, 1399
Primary stimulus, effect of on antibody formation, 1106
Proactinomycin, 178
Probability in relation to assessment of significance, 983 *et seq.*
Probability tables in counting bacteria, 82, 2022
Promin. *See* Sulphone
Prontosil. *See* Sulphonamido-crysoidin
Propamidine, 172
Protein constituents of bacteria, 43
metabolism of bacteria, 55
Proteins, antigenic differences between, 253
effect of chemical treatment on antigenic structure of, 253
relation of to full antigenicity, 260
Protein-sparing effect of carbohydrates in bacterial metabolism, 57
Proteus, **642–651**
classification of, 648
diseases associated with, 1497, 1498, 1500, 1501, 1586
hæmolytic activity of, 645
in relation to putrefaction, 645
to summer diarrhœa, 1586
prevention of swarming of, 644, 1528
Proteus, swarming of, 643–645
hydrophilus, 647
mirabilis, 648
morgani, 642 *et seq.*, **651**
vulgaris, 642 *et seq.*, **649**
in wound infection, 1501
X, 642, 647 *et seq.*, 933, 1853–1858
zenkeri, 648
Protista, 949
von Prowazek-Halberstaedter inclusion bodies in trachoma, 1878
Pro-zone in agglutination, 212
in bacteriolysis, 228
in precipitation. *See* Optimal antigen: antibody ratio, 201 *et seq.*
Pseudoanthrax bacillus. *See Bacillus pseudoanthracis*
Pseudo-lymphocytic choriomeningitis, 1932, 1934
Pseudomonas, **506–512**
group of forming bluish-green pigment, 508
in peritonitis, 1498
in summer diarrhœa, 1586
in suppurative lesions of urinary tract, 1500
in wounds, 1501, 1503
æruginosa. *See Ps. pyocyanea*
caviæ, 512
cyanogena, 512
denitrificans, 512
fluorescens, 506 *et seq.*, **511**
pyocyanea, 506 *et seq.*, **510**
antigenic structure of, 508
differentiation of from *Ps. fluorescens*, 511
pathogenicity of for animals, 508, 511
pigment formation by, 508, 510
variation of, 508, 510
radicicola. *See Rhizobium leguminosarum*
Pseudorabies, 1941
relation of to B, and herpes viruses, 1921
Pseudotuberculosis, 426, 777, 1403, 1404, 1649
cladothrichicha, 1275
differentiation of from plague, 1640
experimental reproduction of in animals 777
of man, 1650
of mice, 461, 1649
of pigs, 1650
of rodents, 1649
of sheep, 468, 1403, 1649
Pseudotuberculous enteritis. *See* Johne's disease
Psittacosis, 1872–1875
in fowls, pigeons and petrels, 1872, 1874
in psittacine birds, 1872 *et seq.*
-like diseases, 1875
Ptomaine poisoning, 1595
Puerperal fever, 1480–1485
anaerobic streptococci in relation to, 1484
chemotherapy in, 1485
Dick test in relation to, 1480
intrinsic and extrinsic infection in, 1481
prophylaxis in, 1483
serum treatment of, 1484
various bacteria in relation to, 1485
vitamin A in relation to, 1194

Pulpy kidney disease of lambs, 1782
Pure cultures of bacteria, methods of obtaining. *See* Cultures, pure
Pyæmia, 1491
in foals, 1404
Pyæmia, portal, 1491
Pyelitis, 1499
Pyelonephritis in cattle, 1500
in man, 1499
Pyobacillosis, chronic, of sheep, 1499
Pyocyanin, 508–510
as respiratory pigment, 54
Pyogenic infections, **1490–1506**
Pyrotherapy in treatment of gonorrhœa, 1460
of syphilis, 1824
Pyridine sulphonamide, 163
Pyrithiamine, 164

Q fever, 1850
Quarter-evil, 1778
Queensland, coastal fever of,
B. tropicus in, 841
Leptospira in, 1834, 1837
7-day fever of, 923, 1837

Rabbits, anaphylactic shock in, 1138
experimental pneumococcal pneumonia of, 1669
infectious fibromatosis of, 1979
infectious mononucleosis of, 1286
infectious myxomatosis of, 1978
infectious papillomatosis of, 1980
labial necrosis of, 479, **1788**
meningo-encephalitis of, 1924
oral papillomatosis of, 1980
pox of, 1896
snuffles in, 1647
syphilis in, experimental, 916, 1165, 1814–1816
natural, 1825
tuberculosis in, 424, 429, 1343
virus-III infection of, 1909. *See also* Chapter 55
Rabies, 1935–1941. *See also* Chapter 55
ascending myelitis in, 1935
due to vampire bats, 1935
fixed virus of, 1936
mode of infection of central nervous system in, 1936
Negri corpuscles in, 1937
Radium, effect of on antibody formation, 1104
on bacteria, 109
Rain, bacteria in, 2013
Ramon precipitin reaction, 202
in standardization of diphtheria antitoxin, 1384
Rat-bite fever due to *Actinomyces muris*, 386, 1276
due to *Spirillum minus*, 925, 1276–1278, **1838**
Ratin bacillus, 736, 1603
Rats, *Brucella* infection of, 1720
experimental pneumococcal pneumonia of, 1669
in ætiology of plague, 1627 *et seq.*
in relation to food poisoning, 1603
in relation to typhus fever, 1845
Rats, in relation to Weil's disease, 1830
increasing immunity of to plague, 1635, 1641
infectious anæmia of, 904, 1797
leprosy of, 404, 440, 1361
otitis media in, 393, 1499
pasteurellosis of, 1639
plague in, 1631, 1638
pneumonia of, 393, 1281, 1499, 1699
tuberculosis in, 426, 429
typhoid of, 1600
" Ray-fungus," 373
Reaction velocity of disinfection, 137. *See also* Disinfection
Reagins, 1157 *et seq.*
Receptors, 196
different orders of, 198
Red-water disease of cattle, 891
Rejuvenation, relation of to lag phase, 84–88
Relapse strains of spirochætes, 1806
Relapsing fever, **1803–1808**
arsenic-fast strains of spirochætes in, 1808
immunity to, 1805
Pfeiffer's phenomenon in, 1806
prophylaxis and treatment of, 1808
relapse strains of spirochætes in, 1806
reproduction of in animals, 914, 1807
transmission of, 1804
Treponema recurrentis in, 913, 1803
named varieties of, 1804
Reproduction of bacteria, 33
Resistance, 981. *See* Immunity
of bacteria to various agents, 101–150
methods of examination for, 359, 366
variations in, in relation to disinfection, 140
Resolution, optical, factors determining, 18
Respiration, bacterial, 46
in relation to growth, 85
Respiratory activity of bacteria in relation to growth, 85
infections, **1653–1686**
Respiratory tract, normal distribution of bacteria in, 1023
normal flora of, 1992
Reticulo-endothelial system, antibodies, formation of, rôle of in, 1102
antiviral immunity, rôle of in, 1232, 1233
in relation to antibacterial immunity 1034 *et seq.*
Reversed anaphylaxis, 1140
Rh factor in human blood, immunological significance of, 1088
Rheumatic fever, **1509–1516**
hypersensitive reactions in, 1511, 1513
relation of to Vitamin C, 1197
streptococci in relation to, 1509–1511
Str. pyogenes in relation to, 1511–1514
virus, as cause of, 1514
Rhinopharyngitis in meningitis, 1434, 1436
Rhinoscleroma, 1789
bacillus of, 667 *et seq.*
Rhizobium, 499–502
leguminosarum, 499–502
Rho-variants (ρ) of *Salmonella* bacilli, 303
of vibrios, 521

Rhodococcus agilis, 627
cinnabareus, 627
rhodochrous, 626
roseofulvus, 627
Riboflavin as bacterial vitamin, 66
Rickettsia, **928–937**
antigenic structure of, 933
classification of, 937
cultivation of, 931, 960
diseases caused by, 1841–1862
habitat of, 929
metabolism of, 931
morphology of, 930
pathogenicity of, 933–936
relation of to *Proteus X*, 1857
resistance of, 932
akamushi. *See R. nipponica*
bovis, 937, 1862
burneti, 929, 932, 936, 1850
canis, 937, 1862
conjunctivæ, 936, 1862
conori, 1848
diaporica. *See R. burneti*
melophagi, 929, 931
mooseri, 929 *et seq.*, 935, 1845
nipponica, 929 *et seq.*, 936, 1849
orientalis. *See R. nipponica*
ovina, 937, 1862
pediculi, 929, 1852
prowazeki, 647, 928 *et seq.*, 934, 1841 *et seq.*
louse-borne type of, 934, 1841
murine type of. *See R. mooseri*
quintana, 928, 936, 1851
rickettsi, 929 *et seq.*, 935, 1846
rocha-limæ, 936, 1844, 1852
ruminantium, 929, 1862
trachomatis, 1879
tsutsugamushi. *See R. nipponica*
wolhynica. *See R. quintana*
Rideal-Walker drop method of standardizing disinfectants, 146
Rieckenberg reaction. *See* Adhesion phenomenon
Rift Valley Fever, 1959
Rinderpest, 1965. *See also* Chapter 55
Ringer's solution, 126
Ring-Impfung, 1907
Rivers, bacteria in, 2015
self-purification of, 2020
Rocky Mountain spotted fever, 935, 1846
Rodella's bacillus III. *See Clostridium tertium*
Rodent diseases, bacteriology of. *See under* Rats, Mice, Guinea-pigs, etc.
Rodent typhoid, 1600
Rodents, plague in gerbilles, ground squirrels, field and multimammate mice, spermophiles and tarbagans, 1637
Rolling disease, 947
Roll-tube method of counting bacteria, 82
Römer's method of performing tuberculin reaction, 1328
of titrating diphtheria antitoxin, 1383
of titrating diphtheria toxin, 1376, 1377
Röntgen rays, effect on bacteria, 108
Rough variants of bacteria, 299
mode of division of, 300
salt-sensitivity of, 299
Roughness in relation to antigenic structure, 277, 283
Roup, 1895, 1896
Rous sarcoma of chickens, 1977
Rovida's tube, 353
Rubella, 1952
Ruys' medium, 1528

Sakushu fever, 1834
Saliva, 1986
bactericidal activity of, 1021
normal flora of, 1986
Salivary calculi due to *Actinomyces*, 1272
gland virus of guinea-pigs, 1943
Salmonella, **702–745**
animal reservoirs of, 1598
antigenic structure of, 710–719, 726–745
antigenic types, fermentative varieties of, 718
antigenic variation of. *See also* Variation
α-β phase, 279, 716
artificial phase, 716
H antigens, 716
M antigen, 718
O antigens, 717
OH → O, 717
smooth → rough, 277, 717
specific-group phase, 278, 716
Vi antigen, 717
X antigen, 718
bacteriophage method of typing of, 719, 1548
biochemical activities of, 706, 718
chemical fractionation of, 280, 720, 724
composite antigens in, 712
d, 712, 743. *See Salm. gaminara*
v, vi, and xii, 712, 717
7, 742. *See Salm. carrau*
cultural characters of, 704
genetic resistance to, 975
habitat of, 703
in birds and animals, 1598–1601
in food poisoning, 1594–1604
in the tonsils, 1524, 1604
in typhoid fever. *See* Chapter 69
morphology of, 704
mucoid colonies of, 704, 705
nomenclature of, 702
pathogenicity of, 721, 1519 *et seq.*, 1584, 1596–1601
production of enterotoxic substances by, 1607
resistance to heat and chemicals, 659, 705, 1527, 1528
summarized description of different species of, **725–744**
toxins of, 721, 723, 1595, 1607
Vi antigens in, 713–715, 717, 1525. *See also* Vi antigen
aberdeen, 709, 715, 741, 1597
abony, 707, 713, 727
abortus-bovis, 707, 713, 730
abortus-canis, 727. *See Salm. paratyphi B*
abortus-equi, 707, 713, 730
abortus-ovis, 707, 713, 730
accra, 736. *See Salm. dublin*
adelaide, 709, 715, 744, 1597

Salmonella aertrycke. *See Salm. typhi-murium*
altendorf, 707, 713, 730
amager, 708, 714, 739
amersfoort, 707, 713, 733
amherstiana, 708, 714, 735
anatum, 708, 714, 739, 1597–1599, 1602
arechavaleta, 713, 730
arizona, 709, 715, 744
ballerup, 709, 715, 743
bareilly, 707, 713, 733, 1526, 1597, 1602
batavia, 740. *See Salm. lexington*
berlin, 732. *See Salm. thompson*
berta, 708, 714, 737
binns, 727. *See Salm. typhi-murium*
bispebjerg, 707, 713, 729
blegdam, 708, 714, 737
bonariensis, 708, 714, 735
borbeck, 715, 741
bovis-morbificans, 707, 714, 734, 1597
braenderup, 707, 713, 733, 1597
brandenburg, 707, 713, 729
bredeney, 707, 713, 730
budapest, 707, 713, 729
california, 707, 713, 729
cambridge, 714, 741
canastel, 714, 738
cardiff, 707, 713, 732, 1597
carrau, 709, 715, 742
cerro, 709, 715, 743
chaco, 708, 736. *See Salm. enteritidis*
chester, 707, 713, 728, 1597
choleræ-suis, 707, 713, 731, 1526, 1597
claibornei, 708, 714, 738
coli, Types I–V, 715, 745
concord, 713, 733, 1597
copenhagen, 727. *See Salm. typhi-murium*
danysz, 708, 736. *See Salm. enteritidis*
dar-es-salaam, 708, 714, 737
derby, 707, 713, 729, 1597
dublin, 708, 714, 736, 1597
duesseldorf, 708, 714, 735
duisburg, 738. *See Salm. gallinarum*
durban, 714, 738
eastbourne, 708, 714, 737, 1597
enteritidis, 708, 714, 723, 735, 1526, 1597
lysogenic strains of, 346
essen, 707, 713, 729, 1597. *See also Salm. enteritidis*
florida, 709, 715, 742
gallinarum, 708, 714, 738, 1597
gaminara, 709, 715, 743
gärtner, 735, 736. *See Salm. enteritidis*
georgia, 713, 733
give, 708, 714, 739, 1597
glostrup, 708, 714, 735
goettingen, 708, 714, 738
grumpensis, 715, 741
habana, 709, 715, 742
hartford, 707, 713, 733
heidelberg, 707, 713, 728
heves, 709, 715, 742
hormæcher, 709, 715, 744
horsham, 715, 742
hvittingfoss, 709, 715, 742
illinois, 708, 714, 741
infantis, 713, 732
inverness, 709, 715, 744
Salmonella italiana, 714, 738
java, 727. *See Salm. paratyphi B.*
javiana, 708, 714, 738, 1597
jena, 735, 736. *See Salm. enteritidis*
kaapstad, 713, 729
kaposvar, 707, 713, 729
kentucky, 709, 715, 743, 1597
kiel, 736. *See Salm dublin*
kirkee, 709, 715, 743
koeln, 713, 729. *See also Salm. dublin*
kottbus, 707, 713, 734
kunzendorf, 707, 731. *See Salm. choleræ-suis*
lexington, 708, 714, 740
litchfield, 708, 714, 735
loma-linda, 714, 737
london, 708, 714, 739, 1597
madelia, 709, 715, 742
manhattan, 708, 714, 734
meleagridis, 708, 714, 740, 1597, 1602
mexicana, 707, 714, 734
mikawasima, 707, 713, 733
minnesota, 709, 715, 743
mississippi, 715, 742
montevideo, 707, 713, 733, 1597, 1602
moscow, 708, 714, 737 1526
moskau, 736. *See Salm. enteritidis*
muenchen, 707, 714, 734, 1597
muenster, 708, 714, 739
napoli, 714, 738
narashino, 708, 714, 735
new-brunswick, 708, 714, 740, 1597
newcastle, 741. *See Salm. senftenberg*
newington, 708, 714, 740, 1597
newport, 707, 713, 734, 1597
niloese, 708, 715, 741
nyborg, 708, 714, 740
odense, 727. *See Salm. paratyphi B.*
onarimon, 708, 714, 737
onderstepoort, 709, 715, 742
oranienburg, 707, 713, 732, 1597, 1602
oregon, 707, 714, 734
oslo, 707, 713, 733, 1597
panama, 708, 714, 738, 1597
paratyphi A, 707, 713, 726, 1526 *et seq.*
paratyphi B, 707, 713, 723, 725, 727, 1526 *et seq.*
paratyphi C, 707, 713, 731, 1526 *et seq.*
pensacola, 714, 737
pestis caviæ, 727. *See Salm. typhi-murium*
pomona, 715, 743
poona, 709, 715, 741
potsdam, 707, 713, 732, 1597
pretoria, 715, 741
pueris, 713, 734
puerto-rico, 734. *See Salm. newport*
pullorum, 708, 714, 738, 1555, 1597
reading, 707, 713, 729, 1597
rostock, 708, 714, 737
rubislaw, 709, 715, 741
saint-paul, 707, 713, 729, 1597
salinatis, 707, 713, 728
san-diego, 707, 713, 728, 1597
schleissheim, 707, 713, 730
schwartzengrund, 713, 730
selandia, 708, 714, 740
sendai, 708, 714, 737

Salmonella shangani, 708, 714, 739
senftenberg, 708, 715, 741, 1597
simsbury, 708, 715, 741
solt, 709, 715, 741
stanley, 707, 713, 728, 1597
storrs, 727. *See Salm. typhi-murium*
sundsvall, 715, 742, 1597
szentes, 709, 715, 743
taksony, 708, 715, 741
tel-aviv, 709, 715, 743
tennessee, 707, 713, 734, 1597
thompson, 707, 713, 732, 1597
tim, 740. *See Salm. newington*
typhi, 708, 714, 722, 735, and Chapter 69
in the tonsils, 1524
typhi-murium, 707, 713, 722, 727, 1597, 1600
typhi-suis, 707, 713, 732
uganda, 708, 714, 739
unnamed type, 714
urbana, 709, 715, 744
vejle, 708, 714, 739
virchow, 707, 713, 732, 1597
virginia, 714, 735
voldagsen, 732. *See Salm. typhi-suis*
weltevreden, 714, 740
wichita, 709, 715, 742
worthington, 709, 715, 742, 1597
zagreb, 707, 713, 729
zanzibar, 708, 714, 740
Salt agglutination, 214
Salt sensitivity of rough variants, 299
Salts, antagonistic effect of, 125
effect of on bacteria, 120 *et seq.*
on other germicides, 127
on pigment formation, 633, 635, 637
mode of action of, 127
susceptibility of Gram-positive and Gram-negative bacteria to, 125
Sampling errors, assessment of, 981 *et seq.*
Sandflies, in relation to *verruga peruana*, 1797
Sandfly fever, 1959
São Paulo typhus, 1847
Saponin solubility of pneumococcus, 571
Saprophytic acid-fast bacilli, 405 *et seq.*
chemical structure of, 409
classification of, 433
cultural characteristics of, 414
effect of glycerine on growth of, 420
pathogenicity of, 428
pigment formation by, 415, 421
Saprophytic viruses, 956
Sarcina aurantiaca, 625, 626
conjunctivæ, 626
conjunctivæ citrea, 626
lutea, 626
mirabilis, 626
ureæ, 626
ventriculi, 625
Satellitism of *H. influenzæ*, 790
Scarlet fever, **1462–1478**
active immunization against, 1473
antitoxic sera, prophylactic treatment with, 1475
standardization of, 1476
therapeutic treatment with, 1475, 1477
Scarlet fever, carriers of, 1469
chemotherapy of, 1477
convalescent serum in, 1477
diagnosis of, 1467
Dick test in, 1078, 1080, 1465, 1467
epidemiology of, 1469
experimental production of, 1464
laboratory infection with, 1464
milk in relation to, 1471, 2039, 2049
"miniature," 1465
mode of spread of, 1471
prophylaxis in, 1472
Schultz-Charlton reaction in, 1465
tonsillitis, relation of to, 1478
treatment of by chemotherapy, 1477
with convalescent serum, 1477
types of *Str. pyogenes* associated with, 1463
Schick test, 1075 *et seq.*, 1376
age groups in relation to, 1076
antibody content of blood in relation to, 1377
blood groups, relation of, to, 1079
conversion rate, 1396
environmental conditions in relation to, 1080
experimental production of diphtheria in positive reactors to, 1085
immunological significance of, 1075–1085
in control of active immunization, 1393–1396
in diphtheria convalescents, 1395
in indigenous populations, 1075–1085
natural immunization in relation to, 1248
Schick dose of toxin in, 1377, 1384
susceptibility of Schick-positive reactors to infection, 1377
Schizomycetes, classification of, 310
Schmitz's bacillus. *See Shigella schmitzi*
Schmorl's bacillus. *See Fusiformis necrophorus*
Schools, diphtheria immunization in, 1248–1251, 1399
Schultz technique in study of anaphylactic shock, 1146
Schultz-Charlton reaction, 1465
Scours, black, 527
white, 667, 1662
Scrapie, 1942
Scrub typhus, 1849, 1856
Scurf staphylococcus, 622, 1997
Sea water, bacteria in, 2015
Seasonal variations in immunity, 1205
Secondary stimulus, formation of antibodies in response to, 1110
Selenium salts 659, 706, 1528
Sensitizer, Bordet's conception of in hæmolysis, 193
Septic sore throat borne by milk 1471, 1478, 2049
Septicæmia, bacteriology of, 1490
hæmorrhagica. *See* Pasteurellosis
Serological reactions, in identification of bacteria, 361. *See also under* Antigen-antibody reactions
Serotherapy. *See under individual diseases*
Serotoxins, in anaphylactic shock, 1143

Serratia marcescens, 636
Serum, diastase in, 453
maltase in, 367, 534
Serum-fast strains of spirochætes in relapsing fever, 1806, 1808
Serum reactions. *See* Antigen-Antibody reactions
Serum sickness, 1152
Seven-day fever of Japan, 1834
of Queensland, 1837
Sewage, activated sludge process of purification, 2033
bacteriology of, 2032
pleuropneumonia-like organisms in, 939, 947
Shake-tube method, use of in obtaining pure cultures, 353
Sheep, anaerobic infections of. *See* Anaerobic infections of animals
anthrax of, 1731
black disease of, 1780
blackleg in, 884, 885, **1778**
blue-tongue of, 1967
braxy of. *See* Braxy
Brucella infection of, 1697, 1706
caseous lymphadenitis of, 459. *See also* Pseudotuberculosis
chronic pyobacillosis of, 1499
contagious agalactia of, 945, 1867
contagious pneumonia of, acute, 779, 1648
contagious pustular dermatitis of, 1897
enzootic hepatitis of, 1959
foot-rot of, 481, 1787
infections of with *Cl. welchii*, 1781
infectious enterotoxæmia, 1782
lamb dysentery, 1781
pulpy kidney disease, 1782
"struck," 1782
infectious necrotic hepatitis of, 1780
Johne's disease of, 1363
joint-ill in lambs, 1284, 1496
lombriz of, 1646
louping-ill of, 1925
Nairobi disease of, 1967
pox of, 1895
pseudotuberculosis of, 1403, 1649
scrapie of, 1942
tuberculosis in, 424, 429, 1343
Shell fish, 2031
Shell fish in relation to enteric fever, 1541
Shiga's bacillus. *See Shigella*
Shigella, **685–699**
antigenic structure of, 687
bacteriophage action on, 693
biochemical reactions of, 686
tabulated, 688
chemical fractionation of, 693
classification of, 696
colonial types of, 686
differentiation of by Michaelis' acid agglutination test, 692
disease due to, 1561–1576, 1584–1585, 1598
hæmolysin formation by, 686
metabolism of, 686
morphology of, 685
nomenclature of, 685, 698
Shigella, pathogenicity of, 695, 1561 *et seq.*
resistance of, 686
smooth-rough or type-group variation in, 686, 690, 692
toxin-formation by, 693
alkalescens, 677, 685 *et seq.*, 1569
dispar, 685 *et seq.*, 1569
flexneri, 685 *et seq.*, 1561 *et seq.*, 1585, 1598
relation of to *Salmonella* group, 692, 744, 745
newcastle, 685 *et seq.*, 1569
paradysenteriæ, 699
para-Shiga group of, 685 *et seq.*, 1568
schmitzi, 685 *et seq.*, 1567
shigæ, 685 *et seq.*, 1566
sonnei, 685 *et seq.*, 1568, 1585
sp. ? boyd Types 170, P288, D1, P274, 685, 688–690, 697–699, 1569
Shop typhus, 1845, 1856
Shwartzman phenomenon, 1176
relation to pathogenesis of infection. 1177
"Side-chain" theory, 196
Side-to-side inoculation, 1231
Silica as kataphylactic agent, 1207
Silicosis, in relation to tuberculosis, 1208
Single cell methods, use of in obtaining pure cultures, 357
Sinusitis, 1499
Site of formation of antibodies, 1101–1106
Size of bacteria, 16
increase in, 841
variation of with age, 84
in relation to opacity, 81
Skin, bactericidal action of, 1019
formation of antibodies in, 1102
local immunity of to streptococcal infection and staphylococcal infection, 1181, 1182, 1184, 1185
normal flora of, 1997
of infants, sensitivity of in relation to Dick test, 1078
staphylococcal and streptococcal infections of, 1492–1493
Slime-fever. *See* Swamp fever.
Smallpox, **1884–1895**
elementary bodies in, 958, 1887
Guarnieri corpuscles in, 1887
immunity in, 1888. *See also* Chapter 55
laboratory diagnosis of, 1887
mode of infection in, 1884
Paschen's granules in, 1887
Paul's test in diagnosis of, 1888
relation of cow-pox and vaccinia to, 1888–1890
reproduction of in animals, 1885
serum against, 1895
types of, classical, 1884
variola minor or alastrim, 1884
vaccination against, 1890
complications of, 1894
foot-and-mouth virus in vaccine, 1894
protective effect of, 1891
Smegma bacillus. *See Mycobacterium smegmatis*
Smooth → Rough (S → R) variation, 299
Smoothness and roughness in relation to immunity, 1055

Smoothness in relation to antigenic structure, 277, 285
Sneeze, droplets in, 2003
Snotsiekte, 1966
Snow, bacteria in, 2014
Snuff, in treatment of nasal diphtheria carriers, 1386
Snuffles in rabbits, 1647
Soft chancre, 1790
Sodium desoxycholate, 571, 659, 706, 1528
Somatic antigens, 276, 283
Sonic waves, effect of on bacteria, 110
Sonne's bacillus. *See Shigella sonnei*
Sordelli's bacillus. *See Clostridium bifermentans*
Species, bacterial, 316
Specific and group phases of flagellar (H) antigens, 278
Specificity of antigen-antibody reactions, 242
Spectrum, diagrammatic representation of, 103
Spinulosin, 178
Spirillum, **527–529**
minus, 529, **925**, 1276–1278, 1838
rubrum, 527, **528**
serpens, 529
undula, 527, 529
volutans, 529
Spirochæta, characters of, 907
morsus muris. *See Spirillum minus*
plicatilis, 907
Spirochætes, 907–925. See also *Spirochæta*, *Cristispira*, *Leptospira* and *Treponema*
general characteristics of, 909–912
in human mouth, 919
Spirochætosis avian, 1808
in animals, 1809
Spirochætosis icterohæmorrhagica. *See* Weil's disease
Spleen, effect of removal on antibody formation, 1103
on *Bartonella* infection, 903, 1797
Spontaneous generation, controversy on, 4
Spore formation, effect of calcium on, 291
variation in, 291
Spores, as antigens, 279, 840
bacterial, 24
bacteriophage in relation to, 347
chromatinic bodies of, 25
germination of, 25
Sporogenes vitamin, 69
Spotted fever, due to *Neisseria meningitidis*. *See* Meningitis
due to *Rickettsia rickettsi*, 935, 1846
Spreading-factors in bacterial infection, 1012
Staining of bacteria, 37
Staining reactions in identification of bacteria, 358
Standard deviation, 985
Standard error, 987
of a difference, 987, 994
Standardization of antitoxin, 238. *See also under individual diseases and organisms*
of disinfectants. *See* Disinfectants
Standardization of various reagents. *See under individual reagents*
Staphylocoagulase, 617
Staphylococcus, **607–622**
antigenic structure of, 613
antiserum to, 620, 1504
bacteriophage typing of, 614
biochemical reactions of, 612
capsulation of, 608
chemical fractionation of, 613
classification of, 620
coagulase formation by, 617
cultural reactions of, 609
enterotoxin of, 616, 1605
habitat of, 608
hæmolysin production by, 615, 616
α-hæmolysin, 615
β-lysin, 616
γ-lysin, 616
in broncho-pneumonia, 1667
in food poisoning, 1604–1608
in suppurative lesions, 1490–1506
leucocidin production by, 616
metabolism of, 610
morphology of, 608
pathogenicity of, 618
pigment production by, 611
resistance of, to heat and disinfectants, 612
satellite growth of *H. influenzæ* in relation to, 790
toxin production by, 614, 1605
toxoid in vaccination, 1506
vaccines of in treatment, 1505
albus, 607 *et seq.*, 620, 622
aurantiacus, 622
aureus, 607 *et seq.*, **621**
in wound infection, 1501–1503
candicans, 622
candidus, 622
citreus, 607 *et seq.*, 620, 622
epidermidis, 622
epidermidis albus, 607
pyogenes albus, 607
pyogenes aureus, 607
pyogenes citreus, 607
salivarius, 607, 622
salivarius pyogenes, 607
Starvation, effect of on resistance, 1201
Stationary phase of growth, 83, 94
Statistical methods, use of, **980–1001**
Sterilization, early work on, 7
of milk, 2053
Stern reaction, 706
Stomach, bactericidal mechanisms of, 1020
normal flora of, 1987
Stomatitis, aphthous, 1897
contagious pustular of horses, 1895
herpetic, 1897
vesicular of horses, 1907
Strangles, 581, 582, 1496
of horses, 1496
Straus's reaction, 489, 490
Streptobacillus moniliformis. *See Actinomyces muris*
Streptococcus, **559–601**
anaerobic species of, 596, 1772, 1996
in gas gangrene, 1772
in puerperal fever, 1484
in septicæmia, 1491
antigenic structure of, 572–588

Streptococcus, bile, effect of on growth of, 572
classification of, 579–589
by changes produced on blood media, 564
by fermentation reactions, 569
growth characters of, 562
growth requirements of, 562
hæmolysis produced by, 564–569
hæmolytic group of, antigenic structure of, 575–588
chemical structure of, 279, 280, 574–577
Group A, 575, 576, 579. *See also Str. pyogenes*
Group B, 575, 576, 578, 580. *See also Str. agalactiæ*
Group C, 575, 576, 578, 581, 594
Group D, 575, 576, 578, 582, 593. *See also Str. fæcalis*
Group E, 576, 578, 586, 594
Group F, 576, 578, 586, 594
Group G, 575, 576, 578, 586, 594
Group H, 576, 587, 594
Group K, 576, 578, 587, 594
Group L, 576, 587, 594
Group M, 576, 587, 594
Group N, 576, 587, 594. *See also Str. lactis*
in normal nasopharynx and tonsils, 1993, 1994
matt and glossy forms of, 301, 577, 595
non-hæmolytic variants of, 567
smooth and rough forms of, 300, 594, 595
heat resistance of, 572, 583
in appendicitis, 1497
in cholecystitis, 1497
in mastitis, 1494
in peritonitis, 1498
in sinusitis, 1499
in suppurative lesions of the urinary tract, 1500
methylene blue, reduction of by different species of, 572
morphology of, 560
motile, 562
pathogenicity of, 590
toxin production of, 590
variation in, 594
acidominimus, 589
agalactiæ, 581, 598, 1494
in mastitis, 1494
pathogenicity of, 592, 1494
bovis, 585, 586, 588
cremoris, 588
durans, 585
dysgalactiæ, 582
epidemicus, relation of to *Str. pyogenes*, 580
equi, 582
equinus, 589
fæcalis group of, 572, 583, 593, 600. *See also* Group D and *Str. fæcalis*
classification of, 583
endocarditis in relation to, 1514
heat resistance of, 572
in water, 2013, 2025, 2028, 2029
relation of to *Str. lactis*, 585
Streptococcus glycerinaceus, 585
hæmolyticus, 559. *See Str. pyogenes*
inulaceus, 585
kefir, 588
lactis, 576, 601
in milk, 2037
relation of to *fæcalis* group, 583–585
liquefaciens, 585
mitis, 589
pneumoniæ, 559 *et seq.*, 592, 598
antigenic structure of, 572
antigenic types of, 1670
transmutation of, 305
antigens of, 279
bile solubility of, 571
capsular swelling reaction of, 241
capsule formation by, 561
chemical structure of, 574
diseases caused by, 1496, 1498, 1499, 1666–1686
frequency of types in health and disease, 1670–1675
hæmolysin of, 568, 592
hyaluronidase production by, 593
in normal nasopharynx, 1993–1994
morphology of, 560
pathogenicity of, 592
polysaccharide antigens of, 574
aggressive effects of, 1071
skin reaction to in pneumonia, 1685
stimulation of antibody formation by, 1119, 1684
Type III, bacterial enzyme acting on, 173, 1682
rough form of, 300, 595
saponin solubility of, 571
typing of in pneumonia, 1678
variations in, 595
pyogenes, 559 *et seq.*, 579, 590, 597
α- and non-hæmolytic colonies of, 567
antigenic structure of, 280, 575–577, 579
capsule formation by, 561
chemotherapy in infections with, 1477, 1485, 1504
erythrogenic toxin of, 591, 1464–1469
in relation to toxæmic infection, 1031
fibrinolysin produced by, 591
hæmolysins of, 566, 590
hyaluronic acid production by, 591
hyaluronidase production by, 591
in wound infection, 1501–1503
infections due to, 1462–1506, 1511, 1666
leucocidin of, 590
local immunity to, 1180–1182
M and T antigens of, 577
pathogenicity of, 590
presence of in normal throat, 1469, 1993–1994
spreading factor of, 591
toxins produced by, 590
typing of, by agglutination, 577
by precipitation, 577
variation in, 594

Streptococcus salivarius, 589
thermophilus, 589
viridans, 564–566
viridans group of, 564–566, 578, **588**, 593, 600
in blood after tooth extraction, 1515
in endocarditis, 1514
zymogenes, 585
Streptolysin, 566, 590
rheumatic fever, in relation to, 1512
Streptomyces, 382
Streptomycin, 178
Streptothricin, 175, 179
Streptothrix. *See Actinomyces and Fusiformis*
cuniculi. *See Fusiformis necrophorus*
maduræ. *See Actinomyces*
moniliformis. *See Actinomyces muris*
muris ratti. *See Actinomyces muris*
Strongyloplasms, 949
Stuttgart dog plague, 1837
Subacute bacterial endocarditis, 1515
Suicide colonies, 670
Sulphadiazine, 157
Sulphaguanidine, 157
Sulphanilamide, 157
Sulphapyridine, 157
Sulphathiazole, 157
Sulphonamide drugs, 157
action of on respiratory enzymes of bacteria, 166
on tissue defences, 1212
antagonizers of, 159, 160, 161, 165
chemotherapeutic activity and chemical structure of, 167
mode of action of *in vitro*, 159, 165
in vivo, 161, 1211
Woods-Fildes hypothesis of, 162
relation of to antibody formation, 1212
to immunity, 1211
resistance, acquired, of bacteria to, 160, 161
Sulphonamido-crysoïdin, 156, 158
Sulphone compounds in chemotherapy, 171
Sulphur bacteria, 61
Sulphur dioxide, effect of on bacteria, 149
Summer diarrhœa. *See* Enteritis of infancy
Sunlight, effect of on bacteria, 102–104
on media, 102
Supersonic waves, effect of on bacteria, 111
Suppurative lesions. *See* Pyogenic infections
Surface plate method of counting, 82
Surface structure in relation to antigenic specificity, 263
Susceptibility to disinfectants in relation to age, 85
Swamp fever, 923, 1836
of horses, 1966
Swine, actinomycosis in, 1271
anaerobic infections of. *See* Anaerobic infections of animals
blackleg of, 1778
botriomycosis in, 1492
Brucella infection of. *See Brucella* infection of swine
erysipelas, 395, **1283–1285**
fever, 1963
hog cholera in, 1963
Swine, influenza, 1661
plague, 1646
pleuropneumonia of, 1964
pseudotuberculosis of, 1650
rouget of, 395
tuberculosis in, 424, 429, 1343, 1348
vesicular exanthema of, 1908
Swineherds' disease 1932, 1934
Symplasmata, 638
Synthetic antigens, 253–258. *See also* Antigens
Syphilis, **1812–1825**
diagnosis of, 1816–1824
by animal inoculation, 1817
by microscopical examination, 1816
by serum reactions, 1817–1824
interpretation of, 1820
immunity to, 1165, 1813
in rabbits, experimental, 916 1814–1816
natural, 1825
prophylaxis and treatment of, 1824
pyrotherapy in, 1824

T antigen of *Salmonella* bacilli, 303
Tabardillo, 1841, 1845
Tears, bactericidal action of, 1025
Teeth, bacteræmia following extraction of, 1515
Tellurite media for diphtheria bacilli, 450, 463, 1374
Temperature. *See also* Disinfection.
on antibody formation, 1204
on antigen-antibody reactions, 200
on bacteria in water, 2016
on bacterial growth, 72
on bacterial growth rate, 91
on hæmolysis, 227
on keeping quality of milk, 2037
external, effect of on resistance to infection, 1202
in identification of bacteria, 360
optimum, use of in obtaining pure cultures, 354
Terms used in description of bacteria, glossary of, 369
of cultural reactions of bacteria, 364–367
of morphological appearance of bacteria, 364
Tetanus, **1746–1767**
absorption and mode of action of toxin in 1752–1755
antitoxin to, 1759
calcium salts in relation to, 1206
cephalic, 1747
diagnosis of, 1758
idiopathic, 1750
immunity to, 1755
acquired, 1757
natural, 1755
in animals, 1767
in man, 1746
epidemiology of, 1746
distribution of tetanus bacilli in nature, 1748
incidence, 1746
incubation period, 1747, 1761
mortality, 1747

Tetanus, in man, prophylaxis and treatment of, 1758–1767
by toxoid, 1765
treatment of by prophylactic injection of antitoxin, 1761
by therapeutic injection of antitoxin, 1762
mode of infection in, 1749
post-operative, 1751
neonatorum, 1746
puerperal, 1746
reproduction of in animals, 1749
splanchnic or visceral, 1747
toxoid, active immunization by, 1765
standardization of, 1767
Tetrathionate broth in cultures from enteric fever, 1528
Therapeutic index, 155
Thermal death point of bacteria, 115
Thermophilic bacilli, 838
clostridia, 872
Thermoprecipitin test in anthrax, 1738
Thiamin, 164
as bacterial vitamin, 64, 66
Thorium X, effect of on antibody formation, 1104
Thread method of standardizing disinfectants, 145
Thrombocytobarin reaction, 912, 1833
Thrushes, plague of, 1968
Thunberg tube, use of in study of oxidation-reduction reactions, 47
Tick fever, 1803. *See also* Relapsing fever *and* Typhus fever
due to *Brucella tularensis*, 1720
due to *Rickettsia*, 1846
due to *Treponema*, 1803
due to unknown cause, 1846
Tick paralysis, 1846
Tick-borne diseases, 1846
Ticks in relation to avian spirochætosis, 1809
to louping-ill, 1925
to Nairobi disease, 1967
to relapsing fever, 1803–1805
to spotted fever, 1846–1848
Timothy-grass bacillus. *See Mycobacterium phlei*
Tissue cultures, formation of antibodies in, 1105
of *Rickettsia*, 931
of filtrable viruses, 959–961
Tissue immunity in relation to local immunity, 1187
Toluol, effect of on antibody formation, 1104
Tonsillitis, 1478
chemoprophylaxis in, 1479
chemotherapy in, 1485
rheumatic fever in relation to, 1511
Tonsils, flora of, 1524, 1604, 1992–1996
Total count of bacteria, 80
Toulon typhus, 1845
Toxæmia, intestinal, 1990
Toxicity, measurement of, 995
Toxigenicity, bacterial, variation in, 306
Toxin, Lf and Lr dose of, 1384
Lo and L+ dose of, 239, 1384
Minimal Lethal Dose of, 238, 1384
Toxin-antitoxin mixture in diphtheria immunization, 1392
Toxin-antitoxin reaction, 238–241. *See also* Antigen-antibody reactions
Toxins, bacterial, 1007–1011. *See also under individual species*
constitution of, 1009
mode of action of, 1010. *See also under various bacteria*
Toxoid, 238
as prophylactic agent in diphtheria, 1392
in tetanus, 1765
Toxoid-antitoxin floccules in diphtheria immunization, 1393
Toxoid-antitoxin mixtures in diphtheria immunization, 1393
Toxophore group, 198
Trachoma, 1878–1880
Bacterium granulosis in, 901, 1878
Rickettsia trachomatis in, 1879
virus origin of, 1879
Transmutation, of bacteria, 305
of fibroma to myxoma, 1980
of papilloma to carcinoma, 1980
Trench fever, 936, 1851
Treponema, 908
anserinum, 911, 914
in avian spirochætosis, 1808
buccalis, 1986
calligyrum, 918
carteri, 1804
cobayæ, 919
cuniculi, 918
in rabbit syphilis, 1825
duttoni, 1804
hispanicum, 1804
intermedium, 1986
kochi, 1804
macrodentium and *microdentium*, 908, 919
novyi, 1804
pallidum, 908 *et seq.*, 915–918
filtrable form of, 1816
in syphilis, 1812
parvum, 908
persicum, 1804
pertenue, 912, 918, 1825
phagedenis, 919
recurrentis, 910, 912, 913
in relapsing fever, 1803
refringens, 918
in syphilitic lesions, 1817
sogdianum, 1804
termitidis, 908
usbekistanicum, 1804
venezuelense, 1804
vincenti, 914
in Vincent's angina, 1810
Trypaflavine, 300. *See also* Acriflavine
Trytophan as bacterial vitamin, 67, 163
rôle of in bacterial nutrition, 65
Tsutsugamushi fever, 1848
Tubercle bacillus. *See Mycobacterium tuberculosis*
Tuberculin, 410, 1327
reaction, 1161, 1300–1302, 1324, 1328
in cattle, 1345
in contact children, 1303, 1307

Tuberculin, reaction, in Johne's disease, 1365
in guinea-pigs, 1326, 1328
methods of eliciting, 1324
relationship of to anaphylaxis, 1161, 1330
Tuberculosis, **1289–1349**
allergy in, 1161, 1324. 1326–1336
relation of to immunity, 1330
bacillæmia in, 1322
borne by milk, 1317, 2038
diagnosis of, 1319
due to bovine tubercle bacillus, 1289, 1311, 1317
epidemiology of, 1291
factors affecting mortality from, 1293
race and civilization, 1296
fatality of infection in early life, 1306
frequency of in human beings, 1290, 1299–1308
heredity in, 975, 1298
immunity in, 1326
in animals, 1342–1349
in cats and dogs, 427, 429, 1342, 1343
in cattle, 1342–1348
in fowls and other birds, 427, 429. **1348**
in goats and sheep, 424, 429, 1343
in guinea-pigs, 425, 429, 1325
in horses, 427, 429, 1343
in mice and rats, 426, 429
in rabbits, 424, 429, 1343
in swine, 424, 429, 1342, **1348**
latent, 1303
mortality from, 1292
of bovine origin, 1317 *et seq.*
of human origin, methods of infection in, 1308
prevention of infection, 1336
prophylactic vaccination against, 1336
pulmonary, 1309 *et seq.*
childhood and adult types, 1314
due to droplet infection, 1312
to dust infection, 1312
endogenous and exogenous infection in, 1316
focal lesions in, 1314
pathogenesis of, 1314
silica as kataphylactic agent in relation to 1207, 1294
therapeutic immunization against, 1341
Tularæmia, 1720
laboratory infections in, 1721
lemming fever, 1721
Tumours, filtrable, 1976–1981
Twort-d'Herelle phenomenon. *See* Bacteriophage
"Two-stage" hypothesis of antigen-antibody reactions, 208
Tyndallization, 7, 114
Typhoid fever. *See* Enteric fever
Typhoid-paratyphoid group. *See Salmonella*
Typhus fevers, **1841–1862.** *See also Rickettsia*
Brazilian spotted fever, 1847
Brill's disease, 1841, 1842
classical type of, 934, 1842, 1845, 1854
classification of, 1852, 1854
diagnosis of, 1853–1858
by complement-fixation test, 1856
Typhus fevers, diagnosis of, by infection test, 1857
by virus neutralization test, 1857
by Weil-Felix test, 1853–1858
exanthematic fever of Marseille, 1848
experimental in guinea-pig, avitaminosis in relation to, 1197
fièvre boutonneuse, 1848
flea-borne, 1845
gaol fever, 1842
Kenya fever, 1848
louse-borne, 1842
Mexican typhus, 1845
mite typhus 936, 1849
murine type of, 935, 1845, 1854
of Lukes in dogs, 1837
pathology of, 1844
prophylaxis and treatment of, 1858
by serum in, 1861
by vaccination, 1859
Q fever, 1850
Rocky mountain spotted fever, 935, 1846
São Paulo typhus, 1847
scrub typhus, 936, 1849, 1856
shop typhus, 1845, 1856
South African tick fever, 1848
tabardillo, 1845
tick-borne, 1846–1848
trench fever, 1851
tropical typhus of Malaya, 1845, 1849, 1855
tsutsugamushi fever, 936, 1848
Weil-Felix reaction in, 1853–1858
Tyrocidin, 174
Tyrothricin, 174

Ulcerative lymphangitis of horses, 1403
Ulcus molle, 1790
Ultrafiltration, 951
Ultramicroscopic viruses. *See* Viruses animal
Ultra-violet light, disinfecting action of, 105
effect of on bodily resistance, 1203
on proteins, 105
on toxins, 105
use of in photomicrography, 18, 952
Undulant fever, **1692–1706**
diagnosis of, 1702
due to *Br. abortus*, 1697
abortion in, 1698
butter and cheese infections in, 1701
contact infections in, 1701
institutional outbreaks of, 1701
laboratory infections in, 1701, 1702
latent infections in, 1699
milk infections in, 1700
tonsillar infections in, 1703
due to *Br. melitensis*, 1693
epidemiology of, in Malta, 1694
in other countries, 1697
eradication of by stopping of goats' milk, 1695
infection of fæces and urine in, 1694, 1703
due to *Br. suis*, 1702
milk in relation to, 1695, 1699, 1700, 1706
prophylaxis and treatment of, 1706

Unimolecular reaction, similarity of disinfection to, 137–140
Unit of antitoxin, 238
Unitarian conception of antibodies, 248, 285
Uracil, 71
Urethra, normal flora of, 1997
Uric acid, fermentation of in differentiation of coli-aerogenes group, 664

V factor in relation to growth of *H. influenzæ*, 790–793
Vaccination, 1888–1895
  complications of, 1894
Vaccine therapy. *See under individual diseases*
Vaccinia, 1884–1895. *See also* Smallpox *and* Chapter 55
  elementary bodies in, 953, 1887
  generalized, in man, 1894
    in rabbit, 1228
  presence of virus in circulating blood, 1233, 1889
Vagina, normal flora of, 1996
Variation of bacteria, 288–307. *See under individual species for variation in each*
  α and β phases of antigens, 279, 716
  as a guide to antigenic structure, 276
  bacteriophage as cause of, 327, 340
  biochemical reactions, in relation to, 292
  colony form, in, 299
  correlated variations, 290
  H → O variation, 276, 291, 717,
  impressed variations, 290
  in relation to enzyme systems, 295
    to epidemic spread, 1260
  in size, 358
    during growth, 85
  induction of, 304
  morphology in, 290
  mucoid variants, 300, 718
  pigment production, in, 299
  ρ variants, 303
  smooth → rough (S → R) variation, 277, 278, 299, 717
    production of by growth in immune sera, 304
  specific and group phases of flagellar (H) antigens, 278, 716
  spore-formation in, 291
  terminology employed in, 288
  transmutation, 305
  virulence in, 303, 306
Varicella, 1901
Variola. *See* Smallpox
Veillon tube, use of in obtaining pure cultures, 353, 752
Verruga peruana, 903, 1796, 1797
Vesicular exanthema of swine, 1908
Vesicular stomatitis of horses, 1907
Vi antigen in *Bacterium coli*, 715
  Pasteurella, 774
  Salm. ballerup, 743
  Salm. hormæchei, 744
  Salm. paratyphi B, 720
  Salm. paratyphi C, 731
  Salm. typhi, 717, 719, 720, 735, 1525, 1547
Viable count of bacteria, 81

Vibrio, 514–527
  antigenic structure of, 519
  biochemical characteristics of, 516
  chemical analysis of, 520
  fermentative classification of, 517
  haemolysin formation by, 518
  pathogenicity of, 522
  resistance of, 516
  variation of, 516, 521, 523
  alkaligenes, 902
  berolinensis, 525
  choleræ, 514 *et seq.*, 523
    disease caused by. *See* Cholera
    selective media for isolation of, 515, 1424
    toxin production by, 518
  danubicus, 526
  Deneke. *See Vibrio typogenus*
  El Tor, 518 *et seq.*, 525, 1419
  fetus, 526, 1720
  Finkler-Prior. *See Vibrio proteus*
  heliogenes, 526
  ivanoff, 525
  jejuni, 527
  Massauah, 526
  metchnikovi, 514 *et seq.*, 525
  Nasik, 516, 526
  paracholeræ, 520
  phosphorescens, 522, 523, 525
  proteus, 523, 525
Vibrio subtilis. *See Bacillus typogenus*, 523, 525
Vibrion butyrique. *See Clostridium butyricum*
Vibrion septique. *See Clostridium septicum*
Vincent's angina, 914, 1810
Virucidal antibodies, natural, 1226
Virulence of bacteria, 1005
  measurement of, 995
  relation of to infectivity, 1260
  roughness and smoothness in relation to, 301
  variation in, 303, 306
Virulins, 1070
Virus diseases, 1869–1981. *See under individual diseases*
  allergy in relation to, 1241
  characterized by catarrhal or generalized infection, 1949–1971
    by lesions of the nervous system, 1915–1943
    by lesions of the skin, 1884–1910
    by tumour formation, 1976–1981
  histopathology of, 1228
  immunity to, 1225–1242
  lymphogranuloma-psittacosis group of, 1869–1880
  mechanical factors in relation to lesions of, 1242
  mechanism of infection in, 1227
  spreading factor in relation to, 1242
Virus infections, mechanisms of, 1227
Virus pneumonias, 1875
  cold agglutinins in, 1877
"Virus" preparations, 1603
Viruses, animal, 949–967
  affinity of for special tissues, 957, 1228
  antigenic structure of, 963, 1240

... of, 959
... due to, 1869 1981. *See also and ... individual diseases*
distribution of in tissues, 1227
habitat of, 956
inclusion bodies formed by, 958, 1228
intracellular position of, 958, 1228
killed, as immunizing agents, 1220
latent infections with, 957, 1226
masked, 1889
metabolism of, 963
morphology of, 954
nature of, 966
pathogenicity of, 965
pneumonia due to, 1875–1877
presence of in circulating blood, 1228, 1889
in leucocytes, 957, 1233
resistance of, 961
saprophytic forms of, 956
size of, tabulated, 955
study of by cataphoresis, 950
by centrifugation, 953
by filtration, 949
by microscopy, 951
by ultrafiltration, 951
Mus-III infection of rabbits, 1909 *See also* Chapter 55
Viscera, bacteria normally present in, 1998
Vitamin B_1 *See* Thiamin
Vitamin requirements of bacteria, 66
sporogenes, 69
Vitamins, influence of on immunity, 1190–1199
synthesis and destruction of by intestinal bacteria, 1991
vitamin A, 1191
vitamin B, 1195
vitamin C, 1196
vitamin D, 1198
Voges-Proskauer reaction, 368, 661
Voldagsen's bacillus. *See Salmonella typhi-suis* var. *voldagsen*
Voles *See* Field mice
bacillus of. *See Mycobacterium muris*
susceptibility of to tubercle bacilli, 427, 430
Volutin granules, 23, 832
in *C. diphtheriæ*,, 449
Vulva, normal flora of, 1996
Vulvo-vaginitis in children, 1455

Warts, common and venereal, 1978
Wassermann test, 1817
interpretation of, 1820 *et seq.*
Water, bacterial flora of, 2012
factors determining, 2013–2021
bacteriological analysis of, 2021–2030
classification of coliform organisms, 2024
coliform count, differential, 2023
presumptive, 2022
examination for fæcal streptococci, *Cl. welchii* and pathogenic organisms, 2025
interpretation of, 2025
plate count in, 2021
Water, bacteriological analysis of, standards of purity in, 2029
diseases carried by, 2001
distilled, effect of on micro-organisms, 117
Leptospira in, 1830, 1834
self-purification of, 2030
supplies in relation to enteric fever, 1541
swimming bath, 2030
Weil-Felix reaction, 1853 1858
Weil's disease, 1823–1834
diagnosis of, 1832
differential diagnosis of from epidemic jaundice, 1833
Leptospira biflexa in, 1831
Leptospira canicola in, 1837
leptospiræ in water in relation to, 1831
leptospiral jaundice in animals, 1837
meningitis in, 1829, 1837
mode of transmission of, 1830
infection of rats in relation to, 1830
occupational incidence of, 1829
prophylaxis and treatment of, 1833
relation of to other leptospiral infections 1834–1837
water-borne, 1830
Welch's bacillus. *See Clostridium*
Whitmore's bacillus. *See Pfeifferella*
Whooping cough, 1662–1666
active immunization against, 1664
diagnosis of, 1663
serum treatment of, 1666
Wilson and Blair's medium, 1528
Winter dysentery of calves, 527
vomiting disease, 1798
Wolhynian fever, 1851
Woods-Fildes hypothesis, 162
Worms, in spread of anthrax, 1732
Wound contamination 1501
Wound infection, 1500–1503
sources of pyogenic bacteria in, 1502–1503
Wright's method of counting bacteria, 89

X factor in relation to growth of *H. influenzæ*, 790–794
nature of, 791
X and V factors in relation to infection, 1208
Xerophthalmia, 1191
X-rays, effect of on antibody formation, 1104
on bacteria, 108

Yaws, 1825
Yellow fever, 1953–1958 *See also* Chapter 55
jaundice due to vaccination against, 1957
jungle type of, 1954
viscerotomy service in, 1954
Yersin type of tuberculosis, 426
Yoghurt, 750

Ziehl-Neelsen stain, in identification of bacteria, 359, 407
Zone phenomenon in agglutination, 212
in specific precipitation, 201
Zopfius, 652
zopfii, 648, 652